Universidade de Aveiro Departamento de Química
2013

Mariana Pôncio da Costa Mendes

Principais compostos odorantes de castas de uvas cultivadas na Polónia

Key odorant compounds of selected grape varieties grown in Poland

Universidade de Aveiro Departamento de Química
2013

Mariana Pôncio da Costa Mendes

Principais compostos odorantes de castas de uvas cultivadas na Polónia

Key odorants of selected grape varieties grown in Poland

Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Bioquímica, ramo de Bioquímica Alimentar, realizada sob a orientação científica de Doutor Henryk Jelén, Professor do Departamento de Ciência dos Alimentos e Nutrição da Poznan University of Life Sciences e de Doutora Sílvia Rocha, Professora Auxiliar do Departamento de Química da Universidade de Aveiro.

o júri

Presidente

Prof. Doutor Erwin Wąsowicz
professor catedrático do Departamento de Ciência dos Alimentos e Nutrição da Poznan University of Life Sciences

Prof. Doutora Renata Zawirska–Wojtasiak
professora catedrática do Departamento de Ciência dos Alimentos e Nutrição da Poznan University of Life Sciences

Prof. Doutora Sílvia Maria da Rocha Simões Carriço (co-orientadora)
professora auxiliar do Departamento de Química da Universidade de Aveiro

Prof. Doutor Henryk Jeleń (orientador)
professor catedrático do Departamento de Ciência dos Alimentos e Nutrição da Poznan University of Life Sciences

agradecimentos

Em primeiro lugar, quero agradecer aos meus pais, avós e à minha irmã, pelo apoio incondicional que me deram durante todo este percurso. Aos meus amigos, pela força que me deram, nos bons e maus momentos. E ao João, por me ter aturado sempre com toda a paciência e carinho.

Quero também agradecer a todos os Professores que contribuiram para o meu processo de aprendizagem. Especialmente à Professora Doutora Sílvia Rocha, ao Professor Doutor Henryk Jelén, que me acompanharam e que me ajudaram imenso durante desenvolvimento da minha tese. Agradeço ao Professor Erwin Wąsowicz e à Professora Renata Zawirska–Wojtasiak pela disponibilidade de participarem na apresentação do meu trabalho.

Por fim, o meu agradecimento à *Poznan University of Life Sciences*, que me recebeu de braços abertos e que me proporcionou um ano repleto de aprendizagem.

~

Firstly, I want to thank my parents, to my grandparents and to my sister, for the unconditional support throughout this journey. To my friends, who gave me the strength, in good times and bad. And to João, for being always there for me, with all the patience and affection.

Furthermore, I'd like to thank all the teachers who contributed to my learning process. Especially to Professor Sílvia Rocha, to Professor Henryk Jelén, who accompanied and helped me immensely throughout the development of my thesis. Thanks to Professor Erwin Wąsowicz and to Professor Renata Zawirska–Wojtasiak for taking the time to attend to our thesis presentation.

Finally, my thanks to the Poznan University of Life Sciences, who received me with open arms and gave me a full year of knowledge.

palavras–chave

Vitis vinifera L., variedades de uvas brancas, compostos odorante chave, extração de fase sólida (SPE), cromatografia de gás–olfatometria (GC–O), análise por diluição do extrato aromático (AEDA), cromatografia de gás–espectrometria de massa (GC–MS).

resumo

Mília, Merzling, Freiminer, Traminer, Jutrzenka e Adalmiina, são algumas das variedades de uvas *Vitis vinifera* L. cultivadas na Polónia, utilizadas para a produção de vinho branco. Este trabalho teve como objetivo a caracterização de compostos odorantes chave nesses tipos de uvas. Para isso, cromatografia de gás-olfatometria (GC-O), utilizando uma abordagem de análise por diluição do extracto aromático (AEDA) e cromatografia de gás-espectrometria de massa (GC-MS) foram aplicadas. A identificação das principais compostos odorantes foi baseada no cálculo e comparação dos índices de retenção (RI). Foram estudados compostos odorantes livres e glicosidicamente ligados. Os componentes de odor mais relevantes identificados foram: *β*-linalol, *cis*-*p*-ment-8-en-1-ol, ácido gerânico, 2,6-hexadienal e isopulegol para uvas Mília; *cis*-*p*-ment-8-en-1-ol, 4-terpineol e *β*-linalol para uvas Merzling; *(E)*-8-hidroxilinalol para uvas Freiminer; 1,1-dimetil-2-propil-ciclo-hexano, geranial, *(-)*-*γ*-elemeno, *p*-menten-9-al, *β*-farneseno e seringaldeído para uvas Traminer; álcool feniletílico, aldeído láurico, neral, 2,6-dimetil-3,7-octadien-2,6-diol e *trans*-2,7-dimetil-3,6-octadien-2-ol para uvas Jutrzenka; álcool feniletílico e 1-(2,3,6-trimetilfenil)-3-buten-2-ona para uvas Adalmiina. Um padrão de odorantes chave específico foi estabelecido para cada uma das seis variedades de uva em estudo.

keywords

Vitis vinifera L., white grape varieties, key odorants, solid phase extraction (SPE), gas chromatography–Olfactometry (GC–O), aroma extract dilution analysis (AEDA), gas chromatography–mass spectrometry (GC–MS).

abstract

Mília, Merzling, Freiminer, Traminer, Jutrzenka and Adalmiina, *Vitis vinifera* L. grape varieties, are some of the grape varieties grown in Poland, used for the production of white wine. This work aimed the characterization of key odorant compounds in these types of grapes. For that, gas chromatography–olfactometry (GC–O), using aroma extract dilution analysis (AEDA) approach, and gas chromatography–mass spectrometry (GC–MS) were applied. Identification of the key odor compounds was based on the calculation and comparison of retention indices (RI). Free and glycosidically-bound odor compounds were studied. The most relevant odor components identified were: *β*–linalool, *cis*–*p*–menth–8–en–1–ol, geranic acid, 2,6–hexadienal and isopulegol for Mília grapes; *cis*–*p*–menth–8–en–1–ol, 4–terpineol and *β*–linalool for Merzling grapes; *(E)*–8–hydroxylinalool for Freiminer grapes; 1,1–dimethyl–2–propyl– cyclohexane, geranial, *(–)*–*γ*–elemene, *p*–menthen–9–al, *β*–farnesene and syringe aldehyde for Traminer grapes; phenylethyl alcohol, lauric aldehyde, neral, 2,6–dimethyl–3,7–octadiene–2,6–diol and *trans*–2,7–dimethyl–3,6–octadien–2–ol for Jutrzenka grapes; phenylethyl alcohol and 1–(2,3,6–trimethylphenyl)–3–butene–2–one for Adalmiina grapes. A specific key odorant pattern was established for each one of the six grape varieties under study.

Main Index

Figure Index

Table Index

List of abbreviations

AEDA	Aroma extraction dilution analysis
CHARM	Combined hedonic response measurement analysis
DHS	Dynamic headspace
FD	Dilution factor
FID	Flame ionization detector
GC	Gas chromatography
GC–MS	Gas chromatography–mass spectrometry
GC–O	Gas chromatography–olfactometry
HS	Headspace
HS–SPME	Headspace solid–phase micro–extraction
MS	Mass spectrometry
NIST	National institute of standards and technology
OAV	Odor activity values
RI	Retention indices
SAFE	Solvent assisted flavor evaporation
SHS	Static headspace
SNIF	Surface of nasal impact frequency
SPE	Solid–phase extraction
SPME	Solid–phase micro–extraction

1 – Introduction

1.1 – Grapes

Grape (*Vitis vinifera* L.) is one of the world's largest fruit crops and is mainly grown for wine production (1). The grape is a non–climacteric fruit and is classified as a berry (2).

The family Vitaceae includes 11 genera and nearly 600 species but only genus Vitis has edible ones and comprises two subgenera, represented on Figure 1: Euvitis, whose species are known as bunch grape, have 38 somatic chromosomes. The number of species belonging to this subgenus depends on taxonomical criteria and they are usually classified by geographical origin; and Muscadinia which have 40 somatic chromosomes and contain only three species, which are endemic to north and central America (3).

Despite the fact that *V. vinifera* and its hybrids are the most cultivated varieties, each country also has its own wild or harvested grapevines. Thus, more than 8000 grape types have been described (3).

Family	Genus	Subgenus	Species (Origin)
Vitaceae	*Vitis*	*Euvitis*	18–28 (America)
			10–15 (Asia)
			vinifera (Europe)
		Muscadinia	*munsoniana* (America)
			rotundifolia (America)
			popenoeii (America)

Figure 1 – Taxonomical chart of grapevine (3).

Mankind has a close relationship with grapes. There are signs of its cultivation around the Mediterranean Sea during the Bronze Age and it is well accepted that *Vitis vinifera* had its origin near the Black and Caspian seas. It has also been reported that grapes were carried around the world as civilization spread. Today, there are grapevines in all the temperate regions of the world and there have been some trials done to introduce them in tropical regions (3).

Sources about grape history have reported that grapes were harvested for consumption as fresh fruit or processed as raisins and in wine production. Today, grapes are still cultivated to obtain the same commodities; thus, approximately 65% of the world's grape crop is used in wine and juice manufacturing, 20% is consumed as fresh grapes, and 10% is used in the production of raisins (3).

1.1.1 – Physical and chemical composition

The structural components of the mature grape berry are shown in Figure 2. Each section of the berry carries a combination of compounds that vary in quantity and in type, which are inherent to each grape varietal of *Vitis vinifera* (4). Grapes are composed of pulp, skin, and seeds (3).

Grape seeds contribute up to 6% of the total weight of the berry. A berry may have zero to four seeds. Seeds contain carbohydrates, nitrogen compounds, oils (oleic and linoleic), minerals, vitamin E, and phenolic compounds. The seeds contain approximately 20–50% of the total polyphenols in the berry, the greatest concentration of tannins (4).

The berry skin contributes up to 20% of the total berry weight. The skin contains the essential anthocyanins required for red wine, along with flavonols and tannins. The skin is high in citric acid and contains benzoic and cinnamic acids. Aromatic substances, aroma precursors, and a small amount of sugar are also present (4).

Grape pulp is the largest component, contributing up to 85% of the total berry weight. Flesh cells have large vacuoles filled with juice, which is a cloudy slightly yellow coloured liquid. Grape juice is an attractive and healthy commodity and its sensorial properties and nutritive value are determined by both chemical composition and particle size, which are highly dependent on grape variety, berry ripeness, and manufacturing process. The chemical composition of grape juice can be broken down into approximate percentages: 79% water; 20% sugars; 0.6% organic acids; 0.2% inorganic material; with a miscellaneous group of 0.5% (4).

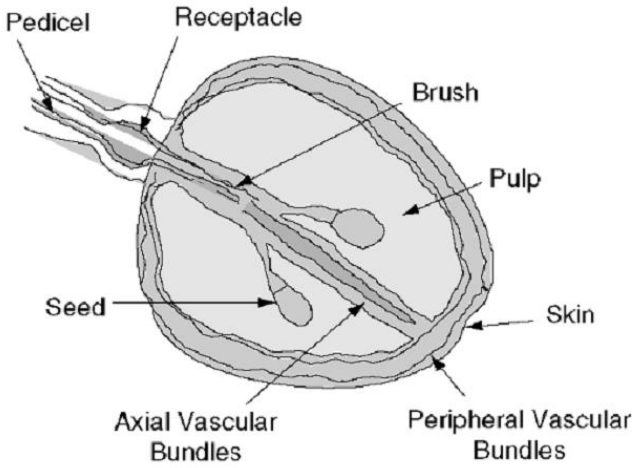

Figure 2 – Grape berry diagram (4).

Bellow, the main composition of the grape is further described.

1.1.1.1 – Carbohydrates

After water, carbohydrates are the most abundant component in grape juice. Both glucose and fructose account for the major part of the total carbohydrate content. Saccharose level is low because it is quickly hydrolysed to glucose and fructose by enzymatic actions (e.g., invertase). Arabinose, ramnose, galactose, xylose, rafinose, or galacturonic acid have been reported at trace levels because they are monomeric constituents of polymeric carbohydrates. Sugar moieties with low polymerized degree or oligosaccharides are usually present as a part of glycoproteins and flavoring or colorant precursors. Grapes have several polymeric carbohydrates such as cellulose, hemicelluloses, and pectin that come from cell walls. The well–established enzymatic cleavage of pectin during berry ripening tends to dissolve some fragments but the remainders stay insoluble. Naturally occurring pectin enzymes break the pectin chain by the cleavage of smooth regions. The other types of arrangements are known as hairy regions, and grapes have no enzymes to break them down. There is no evidence of cellulase and hemicellulase enzymatic activity in grapes, so cellulose and hemicellulose are insoluble due to their huge size (3).

1.1.1.2 – Organic Acids

Grape juice pH varies from 3.3 to 3.8 due to organic acids. Principal acids in grape juice are tartaric acid and malic acid, which constitute more than 90% of the total acidity, although the values vary in a wide range depending on grape variety (3). L–tartaric acid (isomer of tartaric acid in grapes) is found in concentrations of 1–7 g/L, whereas L–malic acid is in concentrations of 1–4 g/L (4). There are many other organic acids in smaller quantities including citric acid, galacturonic acid, which is the major monomer of pectin chains, phenolic and fatty acids, which are originated by hydrolytic cleavage of juice esters (3).

Despite this acidity level, which decreases the pathogen risk, spoilage microbes can grow in grape juice (3).

1.1.1.3 – Nitrogen compounds

Nitrogen containing compounds of grape juice accounts for 20–30% of total grape berry content. It is distributed in inorganic forms (25%), which are mainly ammonium salts, amino acids (70%), peptides (3%) that have mass below 10 kDa, and proteins (2%). The pulp is high in the amino acids leucine, proline, arginine, threonine, and glutamic acid (4). Glycoproteins and enzymes are the main proteins, their size ranging from 10 to 90 kDa. Grape juice contains oxidases such as polyphenol oxidases and peroxidases pectin enzymes like pectin methylesterase and polygalacturonases and also proteases (3).

Total nitrogen consists of all available nitrogen sources and varies greatly between varietals and vineyards. Generally, total nitrogen ranges from 150 to 650 mg/L. The largest concentrations of available nitrogen are derived from the ammonium salts and amino acids (4).

1.1.1.4 – Minerals

Grapes contain a variety of minerals, presented as salts (3) that are divided into two groups: cations and anions (4).

Cations include potassium, sodium, manganese, aluminium, zinc, iron, copper, lead, and calcium. Potassium is the most abundant and has the greatest effect on pH. Sodium and magnesium concentrations are about a tenth of potassium, with calcium being slightly less. The other elements have trace concentrations. Anions consist primarily of phosphate and

sulphate, with trace concentrations of boron, silicon, chlorine, bromine, and iodine. Sulphate accounts for the largest concentration, nearly three times that of phosphate. The other elements occur in trace amounts (4).

1.1.1.5 – Lipids

Grape juice is a poor source of lipids since it has only 1–2% of the grape lipid content. The most abundant types of lipids are phospholipids (65–70%), neutral lipids (15–25%), and glycolipids (10–15%), which have a high content in polyunsaturated fatty acids (3).

1.1.1.6 – Vitamins

Grape juice has greater content of water–soluble vitamins than fat–soluble vitamins and the most important is ascorbic acid. Among the fat–soluble vitamins, grape juice contains only small quantities of carotenoids like lutein and β–carotene, which is a precursor of vitamin A (3).

1.1.1.7 – Odor compounds

Volatile compounds constituting the aroma of fresh grapes fall into several chemical families: terpenes, norisoprenoids, alcohols and polyols, aldehydes, organic acids, esters, methoxypyrazines, sulphur compounds. The greatest contribution to a pleasant olfactory perception of aroma in grapes comes from the group of terpenes and norisoprenoids, while sulphur compounds and methoxypyrazines have a more unpleasant smell, but if carefully balanced add a more distinctive character to some grape varieties (5).

Free volatile compounds in grapes are minor constituents that are predominantly formed through the action of endogenous enzymes, when the grape is crushed and the cell walls are damaged. In addition to free flavor and aroma, grapes contain glycosidically–bound compounds that do not readily contribute to flavor and aroma. These flavorless and non–volatile glycosidically–bound compounds consist of simple single glucosides and disaccharide glucosides, with sugars α–L–arabinofuranose, α–L–rhamnopyranose and β–D–apiofuranose linked to the glucose residue. In grapes, the disaccharide glycosides are dominant. The disaccharides are linked to different aglycones of monoterpenes, norisoprenoids, volatile phenols and other benzene derivatives. The glycosidically–bound volatile compounds are studied by analysing the aglycones released from the non–volatile

precursors through either enzymatic or acid hydrolysis (6). Enzymatic hydrolysis involves cleavage of the glycosidic linkage, and therefore does not induces any further transformation in the chemical structure of the aglycon released. For this reason, enzymatic hydrolysis is generally preferred for the characterisation of the pool of naturally occurring glycosidically–bound volatile compounds. Conversely, acid hydrolysis entails cleavage of the ether linkage between the glucose and the aglycon, resulting in a reactive carbocation that can give a large array of product (7).

- **Terpenes**

The most common flavoring agents in grape juice are terpenes, although its content depends on varieties, which range from 500 to 1700 μg/l (3). The large family of terpene compounds is very widespread in the plant kingdom. Compounds within this family likely to be odoriferous are monoterpenes (compounds with 10 carbon atoms) and sesquiterpenes (15 carbon atoms), formed from two and three isoprene units, respectively. Monoterpenes occur in the form of simple hydrocarbons (limonene, myrcene, etc.), aldehydes (linalal, geranial, etc.), alcohols (linalool, geraniol, etc.), acids (linalic and geranic acid, etc.), and even esters (linalyl acetate, etc.) (8).

About forty terpene compounds have been identified in grapes. Some of the monoterpene alcohols are among the most odoriferous, especially linalool, α–terpineol, nerol, geraniol, citronellol and hotrienol, which has a floral aroma reminiscent of rose essence. The olfactory perception thresholds of these compounds are rather low, as little as a few hundred micrograms per liter. The most odoriferous are citronellol (Figure 3) and linalool (Figure 4) (8).

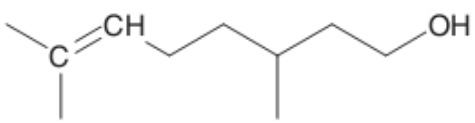

Figure 3 – Chemical structure of citronellol (9).

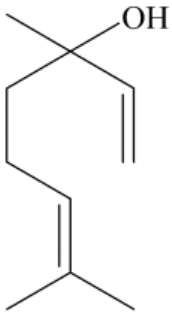

Figure 4 – Chemical structure of linalool (10).

Glycosylated forms of terpenes are frequently more common than the free ones and the relative proportions of free and glycosidically–bound compounds depend on the grape variety (8).

The main monoterpenols and terpene polyols are present in grapes in glycoside form, including the basic "oses": glucose, arabinose, rhamnose and apiose. Four types of glycosides have thus been identified: three diglycosides (6–O–α–L–arabinofuranosyl–β–D–glucopyranoside, 6–O–α–L–rhamnosyl–β–D–glucopyranoside or rutinoside, 6–O–β–D–apiosyl–β–D–glucopyranoside) and one monoglucoside (β–D–glucopyranoside). All grape varieties contain similar glycosides. Among the glycosides corresponding to the most odoriferous aglycones, apiosyl–glucosides and arabinosylglucosides are the most widespread, followed by rutinosides and then β–glucosides.

Grape skins have a higher concentration of free and glycosylated monoterpenes than the flesh or juice. The free terpenol composition varies a great deal in the different parts of grapes, being geraniol and nerol more common in the skin than in the flesh and juice. The proportions of the various bonded terpenols are largely the same throughout the grape (8).

- **C_{13}–norisoprenoids**

The oxidative degradation of carotenoids, terpenes with 40 carbon atoms, produces derivatives with 9, 10, 11 or 13 carbon atoms. Among these compounds, norisoprenoid derivatives with 13 carbon atoms (C_{13}–norisoprenoids) have interesting odoriferous properties and are mainly present in grapes in the form of glycosidically–bound precursors (carotenoids and glucosides) (8).

From a chemical point of view these norisoprenoid derivatives are divided into two main forms: megastigmane and non–megastigmane. Each of these categories include a large number of volatile compounds (8).

The megastigmane skeleton is characterized by a benzene ring substituted on carbons 1, 5 and 6, and an unsaturated aliphatic chain with four carbon atoms attached to C_6 (8).

Megastigmanes are oxygenated C_{13}–norisoprenoids, with skeletons oxygenated on carbon 7 (damascone series) or carbon 9 (ionone series). Among these compounds, β–damascenone (Figure 5), with a complex smell of flowers, tropical fruit and stewed apple is probably present in all varieties of grapes. With its characteristic aroma of violets, β–ionone (Figure 6) is, like β–damascenone, present in all grape varieties (8).

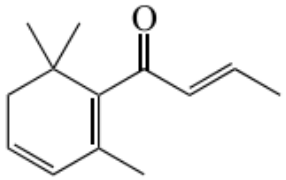

Figure 5 – Chemical structure of β–damascenone (11).

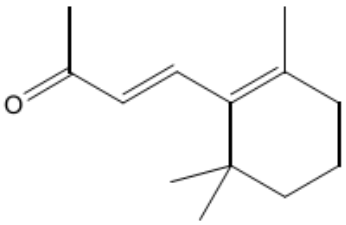

Figure 6 – Chemical structure of β–ionone (12).

Non–megastigmane C_{13}–norisoprenoid derivatives have also been identified, including a few rather odoriferous compounds. The most important of these is TDN (1,1,6–trimethyl–1,2–dihydronaphtalene), which has a distinctive kerosene odor. TDN is generally absent in grapes and young wine, but may appear during bottle aging, as well as actinidols and vitispirane, also in the same family, that have odors reminiscent of camphor (8).

Like monoterpenes, certain C_{13}–norisoprenoids (vomifoliol, 3–oxo–α–ionol, 3–hydroxydamascone) exist in glycosylated form. The currently identified glycosides of C_{13}–norisoprenoids are all monoglucosides. They are not hydrolysed by grape and yeast

glycosidases but they may be revealed by exogenous fungal glycosidases, though the volatile compounds thus released are not highly odoriferous. However, theoretically, in an acid medium, some of them, especially 3–hydroxydamascenone, could produce β–damascenone (8).

- **Methoxypyrazines**

Methoxypyrazines are nitrogenated heterocycles produced by the metabolism of amino acids. Compounds as 2–methoxy–3–isopropylpyrazine (Figure 7), 2–methoxy–3–sec–butylpyrazine and 2–methoxy–3–isobutylpyrazine, have odors reminiscent of green pepper and asparagus, or even earthy overtones and are odoriferous compounds (8).

The compound 2–methoxy–3–isobutylpyrazine was first identified in grapes and, since then, 2–methoxy–3–isobutylpyrazine and then other pyrazines have been identified in many grape varieties and their wine, such as 2–methoxy–3–methylpyrazine and 2–methoxy–3–ethylpyrazine. However, they are much less odoriferous than 2–methoxy–3–isobutylpyrazine (8).

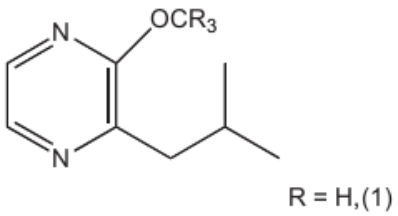

Figure 7 – Chemical structure of 2–methoxy–3–isobutylpyrazine (13).

- **Sulphur compounds**

Sulphur compounds in the thiol family (or mercaptans) are generally held responsible for olfactory defects. However, their major contribution to the aromas of certain fruits and aromatic plants has been clearly established. Two mercaptans, ethyl–3–mercaptopropion–ate and ethyl–2–mercaptopropionate, have been identified as components in the aroma of Vitis labrusca grapes (8).

1.1.2 – Types of grapes cultivated in Poland

Poland is not known as a country of wine producers (14). The wine production is associated mainly with countries of moderate climate with long, hot summers. However, vineyards are located also in countries of cooler climate. In Poland, grapevine was cultivated already in 14th century, first of all by monks for their liturgical purposes. In 2005 the Council of the European Union decided to classify Poland in wine–growing region A (the coldest), similarly to Germany, Austria and the Czech Republic (15).

In recent years there has been a growth of interest in grapevine and winemaking in Poland, mainly due to the emergence of new vine varieties, composed of crossbreeds better suited to the Polish climate. The increased exposure to Western European culture, growth of consumers' knowledge about the dietetic and health properties of wine, the search for new sources of income in Polish agriculture, and the warming of the Polish climate are also responsible for this phenomenon. According to Central Statistical Office, in 2005 there were about 2000 vineyards of a total area of 155 ha in Poland. Since a long time, Zielona Góra, Małopolska, Sandomierz and Podkarpacie have their viticulture and wine production traditions, and wines produced in those regions can compete with alcoholic beverages from traditional wine countries (15). Table 1 describes some of the polish grape varieties used to produce white wine.

Table 1 – Grape varieties used to produce white wine in Poland; some of their characteristics (16).

Name	Origin	Must sugar content (kg/100 L)	Colour	Quality wine	Resistance to disease	Frost resistance
Adalmiina	America	18–22	Yellow–green	Fine	High	–35 ° C
Aurora	France	16–20	Pale yellow	Average	High	–28 ° C
Bianca	Hungary	18–22	Yellow	High	Fine	–25 ° C
Bion	Moldova	15–18	Pale Green	High	High	–25 ° C
Cserszegi Fuszeres	Hungary	16–20	Yellow–green	Fine	Average	–21 ° C
Freiminer	Germany	19–22	Pink	Fine	Average	–23 ° C
Hibernal	Germany	16–22	Pale Green	Fine	Average	–26 ° C

Table 1 (continued)

Name	Origin	Must sugar content (kg/100 L)	Colour	Quality wine	Resistance to disease	Frost resistance
Jutrzenka	Poland	18–22	Yellow–green	High	High	–25 ° C
Merzling	Germany	17–20	Yellow–green	High	Average	–23 ° C
Mília	Slovakia	20–24	Pink	Fine	Average	–20 ° C
Muskat Odessa	Ukraine	18–22	Yellow	High	High	–24 ° C
Nakhodka	Russia	17–20	Pale Green	High	High	–27 ° C
Ortega	Germany	18–22	Pale yellow	High	Low	–21 ° C
Seyval Blanc	France	15–21	Yellow–green	High	Fine	–26 ° C
Serena	Germany	18–20	Yellow–green	High	High	–27 ° C
Sibera	Germany	16–19	Pale Green	High	Fine	–26 ° C
Siegerrebe	Germany	18–22	Pink	High	Average	–22 ° C
Traminer	Austria	16–20	Pink	Fine	Low	–20 ° C
V 71141	Canada	17–20	Pink	Average	Average	–27 ° C
Zenit	Hungary	17–20	Yellow–green	Fine	Low	–18 ° C

1.2 – Food flavor and odor

1.2.1 – Definitions and recognition

Flavor is a key attribute in selection of a particular food product by consumers and, together with texture and appearance, forms the main features that are crucial for consumer acceptance (Figure 8). Although the term flavor combines aroma and taste, the majority of research performed has been related to odorants not the tastants (17).

In–mouth perception of food is complex and is the result of a combination of retronasal aroma, taste and texture perceptions. It is commonly admitted that these three sensory modalities interact one another in different ways. In addition, odor interferes with the other

senses experimented in mouth and makes perception mechanism even more complex to understand (18).

The flavor of food is dependent on an array of volatile compounds – their number, character, and quantities. However, because flavor is related to perception of odorants by our olfactory system, unique features of volatile compounds have to be considered as well, their odor threshold being the most important and features that influence odor thresholds and aroma perception: chirality, concentration, synergistic effects and a type of matrix from which the compounds are released (19).

The flavor of a food will be characterized by odorants which are perceived by the human nose and in the mouth–nose space, respectively. However, flavor descriptors, such as hot, pungent and biting, are also given to sensations received by the general pain, tactile, and temperature receptors in the mouth, nose and eyes (20).

It is only possible to taste and to smell what has been released in the oral cavity during the consumption of the food. Flavoring substances which cause the impressions sweet, sour, salty, bitter and umami must dissolve in the saliva before they can be perceived. Odor substances must reach the oral and pharyngeal cavity in the gaseous phase before they can be smelt (17).

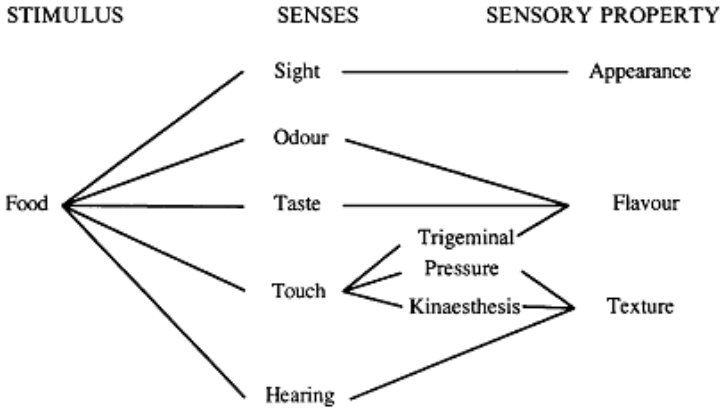

Figure 8 – Relationship of the five senses with sensory properties (17).

As far as olfaction is concerned, it is essential that a strict distinction is made between two types of smelling: nasal and retronasal (17). A scheme of the major structures in the nasal zone is described in Figure 9.

Nasal smelling refers to the “normal” smelling through the nose. The active substances producing the smell pass through the nostrils on their way to the olfactory epithelium in the roof of the nasal cavity and from there through the nasopharynx to the oral/pulmonary cavity from where they are then exhaled. The process corresponds to breathing with the mouth closed. The olfactory perception can be reinforced by sniffing, i.e. by swirling the air intermittently over the ethmoid bone below the olfactory epithelium. The swirling effect causes the active components producing the smell to flow past the olfactory epithelium several times (instead of just once), thereby reinforcing the effect (particularly important in quality testing in the case of weak samples or samples with only very minor differences between them) (17).

Retronasal smelling occurs when an aromatic product is placed in the mouth, the mouth is then closed and the product is “eaten”, causing the active components producing the smell to rise through the nasopharynx to the olfactory epithelium (this can be demonstrated quite simply by holding the nose closed, thus creating a counter–pressure which prevents the active components from rising from the mouth to the nose). This effect can also be reinforced by smacking one’s lips or slurping. This causes additional air to be sucked into the mouth, so that the active parts of the substances producing the smell are carried and swirled past and “round the back of” (= retro) the ethmoid bone (17).

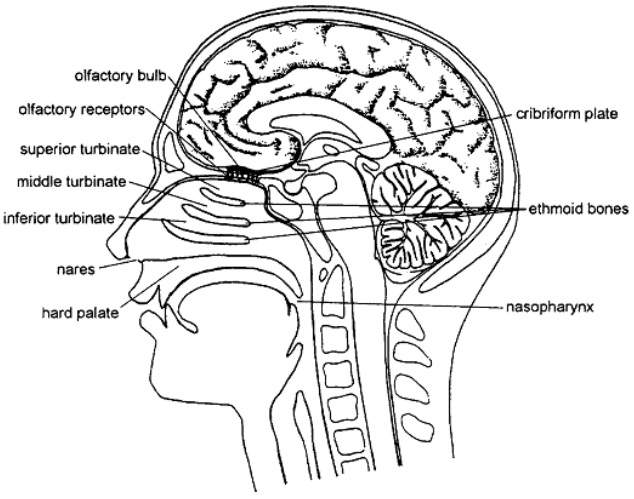

Figure 9 – Human head showing the location of major structures in the nasal area (17).

Different works were carried out in order to study the relation between odor and other sensory perceptions. In previous works, it was shown that the sense of smell and the sense

of taste interfere with each other and that it is hard to perceive these modalities separately. It was demonstrated that congruent odors can enhance taste intensity. For example, caramel odorant could enhance sweetness and decrease sourness; odors such as sardine or bacon could enhance saltiness whereas carrot odor could decrease it. Many studies have been performed to investigate differences between ortho– and retronasal olfaction, however there is little information about their interaction. Finally, many works have been published on the influence of texture on odor perception. A general observation was that the intensity of the olfactory perception decreased when increasing thickness or firmness of the food sample. For instance, it was demonstrated that the intensity of an administered odor decreased when concomitantly eating a semisolid food like custard or a protein gel (18).

1.2.2 – Odorant compound characteristics

Food volatile flavor compounds don't usually exceed 300 Da and represent various chemical classes. Because of their character and molecule size, volatile flavor compounds have been analyzed using gas chromatography (GC) (19).

The amount of volatile substances present in food is extremely low (10–15 mg/kg). In general, however, they comprise a large number of components. Especially foods made by thermal processes, alone (e. g., coffee) or in combination with a fermentation process (e. g., bread, beer, cocoa, or tea), contain more than 800 volatile compounds. A great variety of compounds is often present in fruits and vegetables as well (21). To date, more than 6900 volatiles have been identified in foods and beverages (22).

The human nose perception of volatile compounds, released from foods and fragrances, depends on the extension of the release from the matrix and the odor properties of the compounds. It is known that only a small portion of the large number of volatiles occurring in a fragrant matrix contributes to its overall perceived odor (23) – those compounds that provide the characteristic aroma of the food are, consequently, called key odorants or odor–active compounds (21).

In general, the sensory importance of an odor–active compound depends on its concentration in the matrix, and on its human nose limit of detection. Moreover, the

unpredictable extent of interaction of flavor molecules with each other, and with other food constituents (lipids, protein, carbohydrates, etc.) must also to be considered (23).

Furthermore, these molecules do not contribute equally to the overall flavor profile of a sample (24) and experience shows that many key aroma compounds occur at very low concentrations; their sensory relevance is due to low odor thresholds (23).

Odor threshold is defined as the concentration of a compound in a specified medium that is detectable by 50% of the specified population. Two types of odor thresholds are sometimes distinguished: detection threshold, defined as the lowest physical intensity at which a stimulus is perceptible, and the recognition threshold, which is the lowest intensity in which the stimulus could be correctly defined/identified.

The odor–active compounds are distinguished based on their concentration and odor threshold value – a compound becomes potent odorant when its concentration exceeds its odor threshold. This is achieved either through high concentration in the sample or very low sensory threshold. In Table 2, some examples of aroma compounds with very low odor threshold values are present (19).

Table 2 – Odor thresholds and odor descriptions of some potent food odorants (19).

Compound name	*Odor threshold in water [$\mu g\ l^{-1}$]*	*Odor description*
methional	0.2	boiled potato
β-damascenone	0.002	fruity, sweet
methylthiol	0.02	sulfur, garlic, gasoline
2-acetyl-1-pyrroline	0.1	popcorn
(Z)-1,5-octadiene-3-one	0.0012	geranium-like
2-methyl-3-furanthiol	0.007	boiled meat-like
2-furfurylthiol	0.012	roasted, coffee-like
2-isobutyl-3-methoxy-pyrazine	0.002	hot red pepper

The differentiation between odorants and the remaining volatile compounds has greatly progressed (21), due to the development of odor analysis.

There is no precise definition of what constitutes an olfactory compound. Based on human perception, there are thousands of olfactory substances, spanning a huge range of chemical groups. An odorant compound must be volatile and most compounds have strongly hydrophobic and weakly polar sites. They also tend to bind weakly with cellular constituents, and dissociate readily (25).

1.2.3 – Odor analysis

Progress in instrumental analysis has led to long lists of volatiles (26). The first comprehensive list of volatile molecules present in food matrices comprised a few hundred compounds and, at the beginnings of the 1970s, less than 1500 flavor chemicals had been identified in food products (23).

In the early stages of research in this field, attention was devoted to the development of methods in order to acquire deeper knowledge on the profiles of food volatiles (23). Also, the distinction between odor–active compounds and the whole range of volatiles in a food product was suggested by flavor chemists to be a major task in flavor analysis (22).

The development and application of methodologies for the determination of the chemical composition of aromas and similar mixtures is a challenging task. As a result, chemical characterization of flavor and aroma ordinarily demands state–of–art techniques for sampling and sample preparation, analyte separation, detection and quantification. GC to mass spectrometry (MS) (GC–MS) and other similar detection schemes are the techniques normally employed for flavor and aroma chemical analyses.

The coupling of olfactometric detection to gas chromatography, gas chromatography–olfactometry (GC–O), is also extremely relevant for qualitative and quantitative chemical analysis of fragrances; several instrumental and methodological variants of GC–O are described in the literature, such as combined hedonic response measurement analysis (CHARM) and aroma extraction dilution analysis (AEDA) (27).

Along with the chromatographic techniques, the use of “electronic noses” (arrays of electrochemical sensors that generate an electric signal that emulates the expected response from the human olfactory system) has been growing in recent years. Their more remarkable features, from the analytical standpoint, are the possibility of fast, direct, qualitative and quantitative evaluation of flavor and aroma with limited or no preliminary sample–preparation procedures – although the low sensitivities provided by the devices presently available still prevent their use in solving most flavor and aroma analytical problems (27).

1.3 – Gas chromatography–Olfactometry

GC–O is a valuable method for the selection of odor–active components from a complex mixture. This technique is based on sensory evaluation of the eluate from the chromatographic column, aiming at discovering the odor–active compounds (28) and helping to detect potent odorants, without knowing their chemical structures (26). The role of the detector should be played by a properly educated person or a team of evaluating personnel (28).

A vast number of investigations have been carried out on the flavor of foods, and the introduction of GC–O was a breakthrough in analytical aroma research, enabling the differentiation of a multitude of volatiles in odor–active and non–odor–active (23).

The description of GC modified for the sniffing of its effluent to determine volatile odor activity, was first published in 1964 by Fuller. The GC system was equipped with a non–destructive thermal conductivity detection system with the outlet connected to a sniffing port (also called olfactometry port or transfer line). The latter was located inside a telephone booth, in order to isolate the evaluator from the potential influences of odorants present in the ambient. In 1971, a more sophisticated GC–O system was reported; humid air was added to the GC effluent, thus avoiding nasal mucosa dry–out. Further improvements included the use of a Venturi tube, to maintain capillary column resolution and to deliver, ergonomically, the effluent to the evaluator. Over the following years, the sniffing ports began to incorporate design features and, nowadays, well–planned options are available on the market (23).

GC–O enables the assessment of odor–active components in complex mixtures, through the specific correlation with the chromatographic peaks of interest; this is possible because the eluted substances are perceived simultaneously by two detectors, one of them being the human olfactory system. Therefore, GC–O provides not only an instrumental, but also a sensorial analysis. The latter is defined as the quantification of the human responses to the stimuli perceived by the senses of sight, smell, taste, touch and audition. When coupled to analytical techniques, such as in GC–O, it becomes a precise, descriptive approach to characterise stimuli, evaluating and measuring impressions (23).

As said before, key odorants' sensory relevance is due to low odor thresholds (23). Hence, a large GC peak area, generated by a chemical detector, does not necessarily

correspond to high odor intensities, due to differences in intensity/concentration relationship (24). Consequently, the general interest of researchers was directed to the determination of the contribution of single constituents to the overall flavor of a product (23).

1.3.1 – Overall characteristics of the GC–O technique and instrumentation

In GC–O, qualitative and quantitative evaluation of the odor is carried out for each analyte leaving the chromatographic column. This allows establishing whether a given compound is sensory active at a given concentration and what its smell is, as well as the determination of the time of sensory activity and the intensity of the odor (28).

Figure 10 describes an overall scheme of a GC–O. The design of all commercially available olfactometric ports is very similar. The eluate delivered to the port through a dedicated transfer line is smelled in a glass or a conical port fitted to the shape of a nose. The transfer line is heated to prevent the condensation of semi–volatile analytes on the walls of the capillary. Auxiliary gas (moist air) is added to the eluate to prevent the drying of the nose mucous membranes of the evaluating personnel, as this could cause discomfort, especially in longer analyses. The transfer line length can vary widely, but it has to be long enough to ensure a comfortable sitting position for the evaluator during detection and to avoid discomfort due to the vicinity of hot chromatograph components (28).

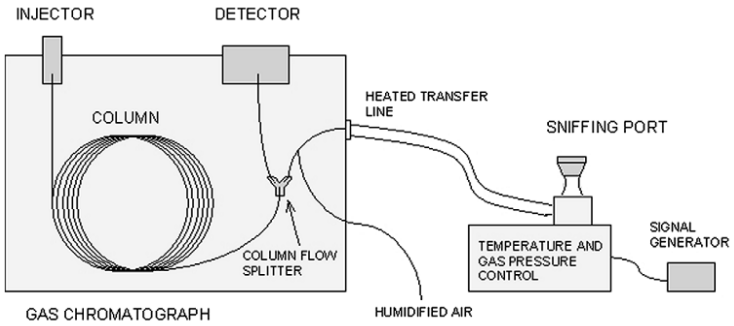

Figure 10 – Gas chromatograph equipped with an olfactometric detector (28).

1.3.2 – Sample preparation for GC–O analysis

A food flavor profile is closely related to the isolation procedure, which should yield a product which is representative of the sample; therefore, the choice of an appropriate sample preparation method becomes crucial (23). Numerous comparative studies revealed that the use of different sample preparation techniques might affect the composition and contents of the isolated compounds (28).

Sample preparation for GC is complicated by a number of factors: the concentration levels of aromatics are generally low, typically in the ppm, ppb, or ppt range – thus, it is necessary not only to isolate the components but also to concentrate them by several orders of magnitude; the volatiles are frequently intracellular and must be liberated by disruption; the sample frequently contains non–volatile components such as lipids, proteins, or carbohydrates, which complicates the isolation process – these components may create problems of foaming and emulsification during isolation procedures and will create artifacts if injected into a hot GC injector port; the aromatic composition of food is frequently very complex and the classes of compounds present cover the range of polarities, solubilities, and pHs; the aroma compounds possess boiling points ranging from well below room temperature to those that are solids, such as vanillin; many components in an aroma are unstable and may be oxidized by air or degraded by heat or extremes of pH (26).

Regardless of which sample preparation technique is employed, it is critically important to assess the organoleptic quality of the isolate. No single technique will prove optimal for every sample, and evaluations should be made to ensure that decomposition and loss of desired components do not occur (26).

Below are described some examples of sample preparation techniques used along with GC–O analysis.

1.3.2.1 – Solid–Phase Extraction (SPE)

SPE is a very popular technique currently available for rapid and selective sample preparation (29) and can be directly applied to isolate odorants from liquid or liquefiable odoriferous samples, such as beverages, fruit pulps and tissues (27).

The principle of SPE involves a partitioning of solutes between two phases, a liquid (sample matrix or solvent with analytes) and a solid (sorbent) phase. This sample treatment technique enables the concentration and purification of analytes from solution by sorption on a solid sorbent and purification of extract after extraction. The general procedure, as described in Figure 11, is to load a solution onto the SPE solid phase, wash away undesired components, and then wash off the desired analytes with another solvent into a collection tube (29).

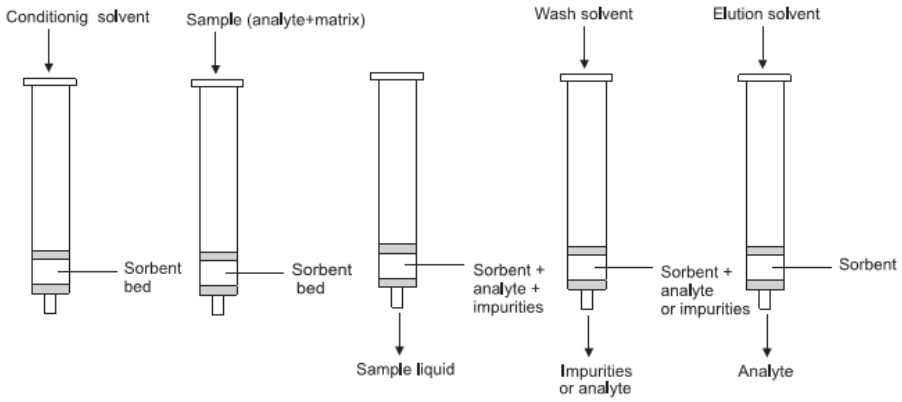

Figure 11 – Solid phase extraction steps (29).

The possibility of using different sorbent phases and eluents makes SPE a very selective technique, and the fact that only minor amounts of organic solvents are used is why SPE has been extensively used for the analysis of volatile aroma compounds and off–flavors. Furthermore, this technique allows the determination of a wide range of volatile compounds, requires small quantities of solvents and short time of analyses. Many benefits of SPE methods have been commonly cited including its robustness, potential for automation, capacity for providing clean extracts, selective isolations and even a fractionation of the different sample components (30).

Many studies based on SPE procedures for monitoring different compounds in wine samples have been published in the last years. SPE has also been successfully used to study the evolution of aromatic compounds of grapes during ripening and to determine the potential aroma in several varieties of grapes (30). Genovese, Lamorte, et al. studied the free and bound volatile compounds of Aglianico and Uva di Troia grape skins and pulp juices, which were extracted and concentrated by SPE technique. In that study, 26 grape aroma compounds were monitored and quantified by GC–MS (31).

1.3.2.2 – Headspace (HS) and Solid–phase micro–extraction (SPME)

HS methods, which are frequently applied to GC–O analysis, may be divided into static (SHS) and dynamic (DHS) headspace; the former is characterised by the sampling of the atmosphere around the HS of a food matrix, located in a vial, after equilibrium has been achieved; the latter removes larger amounts of volatiles due to a constant sweeping of the matrix by a flow of carrier gas (23). Guth and Grosch reported a new concept in aroma research using SHS combination with GC–O. The equipment is composed of a purge–and–trap system for introducing various volumes of gaseous samples without artefact formation, a suitable capillary column, and an effluent splitter to simultaneously perform GC–O and detection by flame ionization detector (FID) or MS. A defined volume of the HS is injected into a precooled trap to focus the volatiles. After flushing the air present in the gas volume, GC separation is started by raising the oven temperature. Dilution steps are made by injecting decreasing HS volumes to evaluate the relative odor potencies (26).

HS has been widely used for analysis of grape and wine volatiles. However, SHS analysis often suffers from poor sensitivity for trace volatiles and DSH analysis suffers from interferences from water and ethanol (32).

SPME is a widely applied solvent–free method which exploits the high adsorption power of a fused silica fibre coated with a specific extraction phase, which is selected according to the type of matrix. The chemical profile of the collected volatiles depends upon the type, thickness and length of the fibre, as well as on the sampling time and temperature (23). A range of fiber coatings are commercially available, providing specificity for a wide range of polar, nonpolar, volatile, and semi–volatile analytes (32).

The main advantages of SPME are simplicity, high sensitivity, small sample volume, and lower cost per analysis. SPME techniques can be successfully applied for polar and non–polar compounds in gas, liquid and solid samples, and can be easily coupled with various analytical instruments such as GC, GC–MS and GC–O (33).

SPME is widely used for analysis of aroma volatiles in many food and beverage matrices. Typically, SPME applications have involved extraction of the volatiles in the HS to avoid interferences from non–volatile matrix components (32). Canuti, Conversano, et al. described a procedure using GC–MS combined with headspace solid–phase micro–extraction (HS–SPME) for profiling the free volatile compounds in Cabernet Sauvignon

grapes. Using this method, 27 flavor compounds were monitored and used to profile the free volatile components in Cabernet Sauvignon grapes at different maturity levels (32).

1.3.2.3 – Solvent Assisted Flavor Evaporation (SAFE)

A further very popular method is SAFE, which may be applied after solvent extraction techniques or be used as an individual extraction method for aqueous foods, such as milk, fruit pulps or matrices with high oil content. This technique removes volatiles under low temperature and high vacuum conditions. The extract is then collected into flasks which are cryogenically cooled with liquid nitrogen. Some attention and time should be devoted to the cleaning of the SAFE apparatus, in order to avoid contamination of liners and columns (23).

Jiang, Fan, et al. studied the free terpenoids in four *Vitis vinifera* varieties by SAFE and gas chromatography–tandem mass spectrometry (GC–MS/MS). In the four varieties of grapes, a total of 30 terpenoids were identified (34).

1.3.3 – GC–O data measurement methods

Over the last decades, GC–O has been largely used in combination with sophisticated olfactometric methods which were developed to collect and process GC–O data, and hence, to estimate the sensory contribution of a single odor–active compound. The choice of the GC–O method is of extreme importance for the correct characterisation of a matrix (23).

Several techniques have been developed to collect and process GC–O data and to estimate the sensory contribution of single odor–active compounds, and can be classified in four categories (35):

- Time–intensity methods, for producing estimates of perceived intensity recorded simultaneously with the elution of the chromatographic peak, e.g. OSME (35);
- Posterior intensity methods, for producing estimates of perceived intensity, which are recorded after a peak has eluted (35);

- Detection frequency methods, for recording detected odors over a group of assessors. The number of assessors detecting an odor (detection frequency) is used as an estimate of the odor's intensity (35);
- Dilution analysis methods, for producing potency values based on stepwise dilution to threshold, e.g. CHARM analysis and AEDA (35).

OSME, a time–intensity measurement, measures the perceived odor intensity of a compound in the GC effluent. The subject rates the aroma intensity by using a computerized 16–point scale time–intensity device and indicates the corresponding aroma characteristics. This technique provides an FID–style aromagram called an osmegram (26).

The posterior intensity method involves the recording of the odor intensity on a scale after a peak has eluted from the column. The method has not been reported in the literature frequently (35).

Detection frequency methods overcome the limitations of a small number of assessors and the use of detection thresholds. The method uses a group of assessors instead of one or two assessors. The number of assessors detecting an odor–active compound at the sniff port simultaneously (the frequency of detection) is used as a measure for the intensity of a compound. A sniffing chromatogram can be composed which cumulates the number of detections of the compound. Usually, the effluent is split for two sniff ports and a flame ionization detector. Thus, two assessors sniff the effluent simultaneously. One analysis, using a panel of ten assessors requires five identical gas chromatographic runs (35).

Dilution analysis is based on successive dilutions of an aroma extract until no odor is perceived by the panellists. This procedure, usually performed by a reduced number of assessors, is mainly represented by CHARM analysis and AEDA (23). Both evaluate the odor activity of individual compounds by sniffing the GC effluent of a series of dilutions of the original aroma extract, and both methods are based on the odor–detection threshold. Several injections are required to reach a dilution of the aroma extract in which odorous regions are no longer detected (26).

AEDA offers an uncomplicated way to assess the importance of particular compounds in overall aroma of the product (14). In AEDA, samples are evaluated by the panellists in increasing dilution order (23). The method is based on the sniffing at olfactometry port compounds from serial dilutions of volatiles extract obtained in a non–destructive way.

Concentrated extract of volatile compound is analysed, then diluted i.e. 2, 4, 8, 16, 32, 64, etc. times, and reanalysed every time (14) and the impact of an odor–active compound is given by its dilution factor (FD) value. The latter is calculated by dividing the largest volume analysed by the lowest volume in which the respective odor–active compound was still detectable. The overall results are reported in an aromagram presenting the FD value, or its logarithm, against the RI, or simply by listing the FD values (23). FD values show in which dilution a particular compound was still perceived at the olfactometry port. This approach provides an insight into the importance of particular compounds into overall aroma. Henryk H. Jelen, Małgorzata Majcher, Mariusz Dziadas et. al, studied the characterization of volatile compounds in Jutrzenka liquor wine, with the emphasis on characterization of compounds responsible for its unique aroma. GC–O was applied to identify the key odorants using aroma AEDA approach. To facilitate free and bound terpenes and C_{13}–norisoprenoids identification SPE was used followed by GC–MS. Among identified key odorants β–damascenone was the compound having the highest FD (4096), followed by isoamyl alcohol, 4–mercapto–4–methyl–2–pentanone (FD = 2048), methional, linalool, ethyl decanoate (FD = 1024) and ethyl hexanoate, furaneol (FD = 512) (14).

On the other hand, in CHARM analysis the dilutions are presented to the panellists in a randomised order, avoiding bias introduced by the knowledge of the dilution being analysed. The panellists record the start and end of each detected odor; the detection duration for each individual is then compiled, and an aromagram is generated by plotting the duration of the odor sensation against the dilution value (23).

The surface of nasal impact frequency (SNIF) method can also be used. During a GC–O acquisition, each panelist continuously smells odors eluting from the chromatographic column and presses a button for the whole duration of the perception of a given odorant. This operation generates, on the computer screen, an olfactogram. After GC–O detection is repeated with the different members of the panel, the resulting individual olfactograms are averaged. Each coincident response of panelists gives a signal, whose height represents the number of panelists having detected an odor at this retention time. After normalization of the mean olfactogram to 100% (100% = peak detected by all panelists), the resulting peak height indicates the detection frequency of this odorant by the panel. Therefore the peak height and its area have been respectively called: NIF (nasal impact frequency) and SNIF

(surface of nasal impact frequency). The SNIF method has been developed to achieve quickness, simplicity, reproducibility and an easy generation of an olfactogram (26).

Other way to quantify the odor sensation and relate it with the amount of detected compound is the concept of odor activity values (OAV), which is a ratio of the amount of detected compound to its odor threshold. This approach relates the concentration of analysed compounds to their sensory importance (14).

1.3.4 – Chromatographic and detection conditions

The choice of proper chromatographic conditions, such as temperature, injection mode and type of the stationary phase of the chromatographic column is very important. Thermal desorption is typically used with HS methods, most often in splitless mode. Solvent extracts, on the other hand, can be injected at low temperature directly on–column, which avoids decomposition of thermally labile analytes. Furthermore, the stationary phase of the chromatographic column should ensure not only high selectivity, but also separation efficiency (28).

As the human nose is the detector used in this technique, it is very important to minimize all factors which can influence the evaluator and consequently affect the analysis. The environment in which olfactometric determination is being carried out is very important, the laboratory must be free of all foreign odors and sounds, and must allow for the maintenance of a consistent temperature and pressure (28).

1.4 – Identification of aroma compounds – gas chromatography–mass spectrometry and retention indices

MS is an analytical technique which involves the production of gaseous ions from the substance under investigation, their separation according to their mass–charge ratio (m/z) and their measurement of relative abundance. It is used to determine the molecular weight

of different compounds by the measurement of their mass to charge ratio and to identify the fragments of a compounds to establish the structure of a molecule (36).

Like a good marriage, both GC and MS bring something to their union. Therefore, it was not surprising that the combination of the two techniques was suggested shortly after the development of GC in the mid–1950s (37). GC has exceptional potential for separating the natural and the synthetic organic mixtures into their components whereas MS provides definite structural information about an organic compound. Due to high sensitivity and fast scan speed of MS, its combination with GC provides a very useful analytical technique for the identification of components eluted from a gas chromatograph (36).

GC–MS has considerable potential in the separation and characterization of food aroma compounds. A great deal of information on flavor compounds has been obtained in the last year for various foodstuffs using GC–MS equipped with various analysers (38).

Identification of volatile compounds based solely on their spectral mass is sometimes difficult, if not impossible. To confirm identifications it is vital to supplement MS results with complementary and independent techniques (39).

One of those techniques is the measurement of the relative retention times of molecules. In this context, the most commonly used parameters are the retention indices (RI). These have been defined by Kovats, in isothermic and isobaric conditions, and by Van Den Dool and Kratz, for temperature programmed chromatography. The most commonly used formula (Figure 13) is the proposed by Van Den Dool and Kratz. This formula allows the calculation of RI (which are generally, but improperly, referred as Kovats indices) (39).

$$RI(i) = 100 \times [t_{R(i)} - t_{R(z)} / t_{R(z+1)} - t_{R(z)}] + 100 \times z$$

Figure 12 – RI formula (39).

In the formula, z is the number of carbon atoms in alkane z, R(i) is the retention time of compound i, R(z) is the retention time of alkane z and R(z+1) is the retention time of the alkane z+1 (39).

RI have the advantage of being fairly insensitive to experimental conditions and can therefore be replicated for a given stationary phase. These indices represent a benchmark measurement providing a common language among chromatographers (39).

Even though several RI databases have been developed, their application to aid molecular identification is not widely employed yet. Two main reasons prohibit the wide usage of the RI values recorded in the current databases. One is that the RI values recorded in the databases may not be reliable. The National Institute of Standards and Technology (NIST) has currently the largest database. In spite of the fact that some erroneous or suspicious RI data were removed from its 2008 version (NIST08), the RI values of some molecules still exhibit a relatively large deviation, of which molecular misidentification in the literature is one of the main causes. Second, compared to the mass spectral database, a relatively small number of retention time data are available. For example, only 21,847 molecules have RI values in the NIST08 database while 192,108 molecules have mass spectra (40).

Columns are classified into three column classes in the NIST08 RI database: standard non–polar, semi non–polar and standard polar column (40). A first classed, called "DB1–like", groups the non–polar phases DB1 (J&W Scientific), HP1 (Hewlett–Packard), SBP1 (Supelco) and BP1 (Scientific Glass Engineering). These phases are composed of poly(dimethylsiloxane) (39). A second class, called "DB5–like", groups the non–polar phases DB5 (J&W Scientific), HP5 (Hewlett–Packard), SBP5 and SE54 (Supelco). These phases are composed of poly(5%–diphenil/95%–dimethylsiloxane) for DB5, HP5 and SPB5 or of poly(5%–diphenil/94%–dimethyl/1%divinylsiloxane) for SE54 (39). A third class, called "CW20M" for Carbowax20 M, groups the polar phases HP–Wax (Hewlett–Packard) and Supelcowax (Supelco) 10. The Carbowax 20M phase is formed from poly(ethyleneglycol) (39).

1.5 – Aims of the thesis

As there aren't many studies characterizing the odor profile and the key odorants of Mília, Merzling, Freiminer, Traminer, Jutrzenka and Adalmiina grapes, it is interesting to proceed to its investigation in order to know which compounds contribute to their aroma profile.

Having that in mind, the main objective of this work was to characterize these grape varieties by identifying the main volatile compounds present in a free and glycosidically-bound form and characterizing these compounds using GC-O and GC-MS.

2 – Methodology

2.1 – Grape varieties

Six types of polish grape varieties were analysed: Merzling, Mília, Freiminer, Traminer, Jutrzenka and Adalmiina. All the varieties were obtained from Golesz wineyard in Jasło region, Podkarpacie, in the 2011 harvest, for wine production (technological maturity). All of these grapes are used to produce white wine. Around 1,5 kg of each grape variety were collected for analysis.

2.2 – Grape juice preparation

After defrosting and destemming the grapes, 1–1,5 kg of grapes were weighted and submerged in distilled water.

The grapes were homogenized using a MPW–120 and a CAT Undrive X 1000 homogenizer, in order to obtain a homogenized must.

After homogenization, the grape juice was subjected to two centrifugations, the first one at 4000 rpm for 15 minutes and the second one at 15000 for 10 minutes. Vacuum centrifugation was sometimes used instead of the second centrifugation, due to equipment non–availability. After centrifugation, a clear homogenized grape juice, ready for analysis, was obtained.

2.3 – Extraction and preparation of the volatile extracts

2.3.1 – Solid–phase extraction

Aromatic compounds appear as much in free form (volatile) and, therefore, with a direct contribution to the aroma, as in glycosidically–bound form (non–volatile). In order to liberate the aglycones, a chemical and/or enzymatic hydrolysis of the bound forms must be performed (41).

SPE was used in order to extract the non–polar (free) and polar (glycosidically–bound) compounds from the grape juice. It was used a Supelco Visiprep SPE station, and Bond Elut C18, 500 mg, 6 mL cartridges (Agilent Technologies).

First, cartridges were preconditioned with methanol, and then with deionized water (3 mL/min, pressure 0,67 atm). Then the grape juice (120 mL) was added to the columns, and afterwards the columns were once again washed with deionized water.

The non–polar compounds were eluted using a mixture of pentane (Sigma–Aldrich) and dichloromethane (Sigma–Aldrich) 2:1 (v/v) (20 mL). The polar compounds were then eluted using methanol (Sigma–Aldrich).

2.3.2 – Non–polar extract treatment

The non–polar extract was placed in a vial with disodium phosphate (Na_2HPO_4), so that all the water is absorbed. Finally, the non–polar extract was concentrated to approximately 0,5 mL, using a rotary evaporator at 40ºC.

The non–polar extract was prepared to be submitted to chromatographic analysis.

2.3.3 – Polar extract treatment – hydrolysis of glycosidically–bound compounds

2.3.3.1 – Enzymatic hydrolysis

The polar extract was dried under a stream of nitrogen (N_2) at 40ºC. Then, the sample was rehydrated using a small portion of Mc'Ilaine buffer pH 5,5 (citric acid 0,1 M and Na_2HPO_4 0,2 M, pH controlled using an Elmetron CP–411 pH meter).

To perform the enzymatic hydrolysis a commercial enzyme preparation, Rapidase AR 2000 composed of pectinases with glycosidases side activities, was used, obtained from DSM Company. To the polar extract 1g of the enzyme diluted in 50 mL Mc'Ilaine buffer pH 5,5 was added. The enzymatic hydrolysis took place during 21h at 40ºC.

After 21h the vial was vortexed in order to denature the enzyme. The hydrolyzed preparation was then again submitted to SPE, and the free compounds were eluted with a mixture of pentane dichloromethane 2:1 (v/v). The sample was then concentrated to approximately 0,5 mL in a similar way as the non–polar fraction.

2.3.3.2 – Acid hydrolysis

The polar extract was dried under a stream of nitrogen (N_2) at 40ºC. Then, the sample was rehydrated using a small portion of Mc'Ilaine buffer pH 2,5 (citric acid 0,1 M and Na_2HPO_4 0,2 M, pH controlled using an Elmetron CP–411 pH meter).

Acid hydrolysis was performed during 1 h at 100ºC. Then the vials were cooled down, the hydrolyzed preparation was again submitted to SPE, and the free compounds were eluted with a mixture of pentane dichloromethane 2:1 (v/v). The sample was then concentrated to approximately 0,5 mL in a similar way as the non–polar fraction.

2.4 – Gas chromatography – olfactometry

GC–O was performed on an HP 5890 gas–chromatograph equipped with an olfactometry port, using a SPB–5 (30m×0.53mm×1.5µm) capillary column. The GC was equipped with a Y splitter dividing effluent between olfactometry port with humidified air supply, and a FID. The operating conditions were the following: initial oven temperature 40ºC (1 min), then 5ºC/min to 180ºC and 25ºC/min to 280ºC.

For all peaks and flavor notes, retention indices were calculated in order to compare the obtained results with GC–MS results and with literature data. RI were calculated for each compound using homologous series of C_5–C_{24} n–alkanes.

The FD of each of the odorants was determined by AEDA. The dilutions used in GC–O experiments were 2, 4, 8, 16, 32, 64, 128 and 256.

2.5 – Gas chromatography–mass spectrometry

After being submitted to GC–O, all samples were analysed in a GC–MS in order to identify their key–odorant compounds.

GC–MS analysis was performed using a 7890A gas chromatograph coupled to a 5975C VL MSD with triple axis detector TAD quadrupole mass spectrometer (both from Agilent Technologies, Santa Clara, CA). A fused capillary column DB–5 MS, (30 m × 0.25 µm ×

0.25 µm, J&W, Folsom, CA) was used for compounds separation (He flow 0.8 ml/min). Temperatures of heated zones were following: transfer line – 280°C, injector – 240°C, oven was programmed at 45°C (2.25 min), then 40°C/min to 300°C (3 min), ion source – 230°C. Spectra (70eV) were acquired in a range of 33–383 Da. For the identification of compounds MSD Chemstation ver. E.02.00 search engine, AMDIS ver. 2.65, NIST 05 library and the AMDIS–created library based on RI were used.

2.6 – Data analysis

After the analysis of the samples by GC–O, the odor description and the FD's in which the odorant compounds were perceived was noted, and their RI were calculated by the formula described in section 1.4.2.3. Then, after GC–MS analysis, the RI of the identified compounds were calculated in the same way. Finally, the RI obtained by GC–O and the RI obtained by GC–MS were compared, in order to obtain a plausible identification for each odorant compound sensed during GC–O analysis.

The results are shown and discussed in section 3.

3 – Results and discussion

Following are the results obtained after the sample's analysis, for the identification of the sensed compounds in the analyzed grapes. The results are divided by grape variety, and then subdivided in three categories, defined as non–polar extract – the compounds present in their free form –, extract after enzymatic hydrolysis and extract after acid hydrolysis – the compounds present in a glycosidically–bound form. The compound's identification followed three criteria: (a) tentatively identified based on mass spectra comparison with NIST library; (b) comparison of RI with the RI of the genuine standard of the identified compound; (c) tentatively identified based on RI comparison using the Flavornet database (http://www.flavornet.org/). After listing the compounds sensed in each of the six grape varieties, their key odorants are selected based on their FD's.

Afterwards, an analysis of the similarities between the free and glycosidically–bound extracts is made, followed by an assessment of the odor description and of the FD in which the odor compounds were sensed. Lastly, an analysis is done about the discovered key odorant compounds' relevance in general grapes.

Attached to this work, are the GC-MS chromatograms of the analysis of the polar and non-polar extract of the six grape varieties.

3.1 – Odorant and key compounds sensed during GC–O analysis

3.1.1 – Mília

Table 3 – Identification of the odorant compounds sensed in the non–polar extract of Mília grapes during GC–O analysis by correlation with the obtained GC–MS results.

RI GC–O	RI GC–MS	Compound	Odor descriptor	FD
783.8	789 [(c)]	1–hexenol [(c)]	Fruity, grassy, sweet	4

(Table 3 – continued)

RI GC–O	RI GC–MS	Compound	Odor descriptor	FD
816.0	817 [c]	butyl acetate [c]	Flowery, grassy	1
839.2	836.0	2,4–dimethyl–1–heptene [a]	Flowery, grassy	2
862.4	865.4	2–hexen–1–ol [a] [b]	Flowery, grassy	1
909.8	919 [c]	2,4–hexadienal [c]	Fruity, ripped, sweet	16
1021.6	1022.6	phenylethanal [a]	Flowery, bitter	4
1029.4	1030.0	2–hexenoic acid [a]	Fruity, sweet	1
1048.1	1045	*cis*–linalool oxide [a] [b]	Flowery, sweet	1
1054.3	1050.9	*β*–linalool [a]	Fruity, sweet	32
1066.8	1058.2	phenylethyl alcohol [a]	Flowery, bittersweet	2
1074.5	1075.6	isopulegol [a]	Fruity, sweet	8
1093.2	1091.0	*cis*–*p*–mentha–8–en–1–ol [a]	Pepper, intense	32
1258.6	1254 [c]	isogeraniol [c]	Flower, citrus	1
1270.9		unidentified	Flowery, bittersweet	1
1293.4	1287.6	geranic acid [a]	Fruity, sweet	16
1417.2	1410.0	*α*–ionone [a]	Fruity, sweet	4
1434.7	1437 [c]	linalyl butyrate [c]	Fruity, sweet	2
1445.8	1445 [c]	*β*–farnesene [c]	Fruity, sweet	1
1469.7	1465.3	2,6,6–trimethy1–2–hydroxycyclohexylidene)–acetic acid lactone [a]	Fruity, sweet	2
1482.5	1481.5	dodecanoic acid [a]	Fruity, sweet	2
1628.9	1629 [c]	*(E)*–whiskey lactone [c]	Flowery	1
1643.4	1644.2	hexadecane [a]	Flowery	1

(Table 3 – continued)

RI GC–O	RI GC–MS	Compound	Odor descriptor	FD
1695.4	1696.1	1–octadecene [(a)]	Fruity, flowery, sweet	1
1896.5	1899.3	9–cedranone [(a)]	Sweet	1
2031.7	2031.4	*(E)*–9–octadecen–1–ol [(a)]	Flowery, sweet	1
2089.3	2085 [(c)]	hydroxycalamenene [(c)]	Flowery, sweet	1

(a) Tentatively identified based on mass spectra comparison with NIST library;

(b) Comparison of RI with the RI of the genuine standard of the identified compound;

(c) Tentatively identified based on RI comparison using the Flavornet database (http://www.flavornet.org/).

Table 4 – Identification of the odorant compounds sensed in the extract obtained after acid hydrolysis of Mília grapes during GC–O analysis by correlation with the obtained GC–MS results.

RI GC–O	RI GC–MS	Compound	Odor descriptor	FD
993.7	1007.3	*cis*–limonene oxide [(a)]	Alcohol	1
1057.4	1056.8	phenylethyl alcohol [(a)]	Flowery, bittersweet	1
1087.0	1086.3	trans–2,7–dimethyl–4,6–octadien–2–ol [(a)]	Fruity, medicine, alcohol	1

(a) Tentatively identified based on mass spectra comparison with NIST library;

(b) Comparison of RI with the RI of the genuine standard of the identified compound;

(c) Tentatively identified based on RI comparison using the Flavornet database (http://www.flavornet.org/).

In Mília grapes, several odor compounds were perceived, with a FD range from 1 to 32.

According to Table 3, the key odor compounds perceived on the non–polar extract of Mília grapes are *β*–linalool (FD=32), *cis*–*p*–menth–8–en–1–ol (FD=32), geranic acid (FD=16), 2,4–hexadienal (FD=16), and isopulegol (FD=8).

No sensations were recorded during GC–O analysis of the extract obtained after enzymatic hydrolysis. Furthermore, according to Table 4, the odor compounds sensed on

the extract obtained after acid hydrolysis, *cis*–limonene oxide, phenylethyl alcohol and *trans*–2,7–dimethyl–4,6–octadien–2–ol, have a FD=1, which implies that they are probably not key odorants on Mília grapes.

Regarding this information, the key–odorants identified in Mília grapes are *β*–linalool, *cis*–*p*–menth–8–en–1–ol, geranic acid, 2,4–hexadienal, and isopulegol.

3.1.2 – Merzling

Table 5 – Identification of the odorant compounds sensed in the non–polar extract of Merzling grapes during GC–O analysis by correlation with the obtained GC–MS results.

RI GC–O	RI GC–MS	Compound	Odor descriptor	FD
927.8	983.1	hexanoic acid [(a)]	Fruity, sweet	2
1049.6	1050.2	*β*–linalool [(a)]	Fruity, sweet	8
1083.9	1088.3	4–terpineol [(b)]	Sweet	64
1094.8	1097.8	*cis*–*p*–mentha–8–en–1–ol [(a)]	Pepper	128
1293.4	1296.5	decanoic acid, ethyl ester [(a)]	Flowery, sweet	1
1485.7	1483.3	dodecanoic acid [(a)]	Fruity, sweet	1

(a) Tentatively identified based on mass spectra comparison with NIST library;

(b) Comparison of RI with the RI of the genuine standard of the identified compound;

(c) Tentatively identified based on RI comparison using the Flavornet database (http://www.flavornet.org/).

Table 6 – Identification of the odorant compounds sensed in the extract obtained after enzymatic hydrolysis of Merzling grapes during GC–O analysis by correlation with the obtained GC–MS results.

RI GC–O	RI GC–MS	Compound	Odor descriptor	FD
783.9	789 [(c)]	1–hexenol [(c)]	Fruity, grassy, sweet	1

(Table 6 – continued)

RI GC–O	RI GC–MS	Compound	Odor descriptor	FD
930.8	938 [(c)]	2–ethylpyridine [(c)]	Flowery	1
1045.0	1040.1	tetramethylpyrazine [(a)]	Sweet, burnt	1
1068.3	1056.8	phenylethyl alcohol [(a)]	Flowery, bittersweet	1
1085.4	1085.3	epoxylinalool [(a)]	Fruity, grass, sweet	4
1295.4	1281.8	*(E)*-hydroxylinalool [(a)]	Flowery	1
1418.8	1422.4	geranyl acetone [(a)]	Fruity, flowery	1
1474.5	1478 [(c)]	linalyl isovalerate [(c)]	Flowery, sweet	1
1617.4	1620.5	3–oxo–*α*–ionol [(a)]	Chemical	1
1878.8	1870 [(c)]	hexadecanol [(c)]	Flowery, sweet	2
2067.7		unidentified	Fruity, sweet	1

(a) Tentatively identified based on mass spectra comparison with NIST library;

(b) Comparison of RI with the RI of the genuine standard of the identified compound;

(c) Tentatively identified based on RI comparison using the Flavornet database (http://www.flavornet.org/).

Table 7 – Identification of the odorant compounds sensed in the extract obtained after acid hydrolysis of Merzling grapes during GC–O analysis by correlation with the obtained GC–MS results.

RI GC–O	RI GC–MS	Compound	Odor descriptor	FD
1013.8	1014 [(c)]	hexyl acetate [(c)]	Flowery	1
1048.1	1049.4	propenethiol [(a)]	Fruit, ripped, sweet	1
1057.4	1056.8	phenylethyl alcohol [(a)]	Flowery, bittersweet	1
1083.9	1091.7	*p*–menthen–9–al [(a)]	Fruity, sweet	2
1229.9	1227.2	geraniol [(a)]	Very nice scent, flowery, perfume, sweet	1

(Table 7 – continued)

RI GC–O	RI GC–MS	Compound	Odor descriptor	FD
1270.9		unidentified	Flowery, bittersweet	2

(a) Tentatively identified based on mass spectra comparison with NIST library;

(b) Comparison of RI with the RI of the genuine standard of the identified compound;

(c) Tentatively identified based on RI comparison using the Flavornet database (http://www.flavornet.org/).

In the three extracts of Merzling grapes, several compounds were perceived, with a FD range from 1 to 128.

According to Table 5 and, the key odorant compounds perceived on the non–polar extract of Merzling grapes are *cis*–*p*–menth–8–en–1–ol (FD=128), 4–terpineol (FD=64) and *β*–linalool (FD=8).

The odor compounds sensed on the extract obtained after enzymatic hydrolysis of Merzling grapes (Table 6) have low FD, from 1 to 4, being epoxylinalool the only compound with FD=4. Furthermore, on the extract obtained after acid hydrolysis of Merzling grapes (Table 7), only compounds with a FD range from 1 to 2 were perceived, being *p*–menthen–9–al the only compound with FD=2.

Regarding this information it is possible to say that, for Merzling grapes, the major odor contributions come from their non–polar extract, by action of *cis*–*p*–menth–8–en–1–ol (FD=128), 4–terpineol (FD=64) and *β*–linalool (FD=8).

3.1.3 – Freiminer

Table 8 – Identification of the odorant compounds sensed in the non–polar extract of Freiminer grapes during GC–O analysis by correlation with the obtained GC–MS results.

RI GC–O	RI GC–MS	Compound	Odor descriptor	FD
771.3	776 [(c)]	isobutyl acetate [(c)]	Flowery, green	2
1068.3	1056.6	phenylethyl alcohol [(a)]	Flowery, bittersweet	1

(Table 8 – continued)

RI GC–O	RI GC–MS	Compound	Odor descriptor	FD
1085.4	1085.3	epoxylinalool [a]	Fruity, grass, sweet	1
1215.6	1213.3	*(R)*–citronellol [a]	Fruity, sweet	1
1252.5	1250.4	cuminol [a]	Fruity, sweet	4
1256.5	1254 (c)	isogeraniol [c]	Flowery	1
1480.9	1481.6	dodecanoic acid [a]	Fruity, sweet	2
1493.6	1495.6	dodecanoic acid, ethyl ester [a]	Fruity, sweet	1
1884.7	1887.9	3,5–dimetoxy–4–hidroxycinnamaldehyde [a]	Flowery	1
2038.9	2031.0	oleyl alcohol [a]	Flowery, grass, sweet	4

(a) Tentatively identified based on mass spectra comparison with NIST library;

(b) Comparison of RI with the RI of the genuine standard of the identified compound;

(c) Tentatively identified based on RI comparison using the Flavornet database (http://www.flavornet.org/).

Table 9 – Identification of the odorant compounds sensed in the extract obtained after enzymatic hydrolysis of Freiminer grapes during GC–O analysis by correlation with the obtained GC–MS results.

RI GC–O	RI GC–MS	Compound	Odor descriptor	FD
700.1	711 [c]	pentanone [c]	Fruity	1
788.0	789 [c]	1–hexenol [c]	Fruity, grassy	2
918.8	915.0	cyclopentane, 1,2,3,4,5–pentamethyl– [a]	Fruity, sweet	1
1001.4	999.1	trimethylpyrazine [a]	Fruity, sweet	1
1049.6	1050.4	*β*–linalool [a]	Fruity, sweet	1
1057.4	1056.8	phenylethyl alcohol [a]	Flowery, bittersweet	1
1077.6	1080.0	pinocarveol [a]	Fruity, sweet, grass	1

(Table 9 – continued)

RI GC–O	RI GC–MS	Compound	Odor descriptor	FD
1088.5	1085.5	epoxylinalool [(a)]	Fruity, sweet	1
1281.1	1284.1	*(E)*–8–hydroxylinalool [(a)]	Flowery, bittersweet	16
1472.7	1478 [(c)]	linalyl isovalerate [(c)]	Flowery, sweet	1
1820.0	1814 [(c)]	*(+)*–nootkatone [(c)]	Flowery, sweet	1
1884.7	1887.9	3,5–dimetoxy–4–hidroxycinnamaldehyde [(a)]	Flowery, sweet	1
2074.9	2080.0	*α*–farnesol [(a)]	Fruity	1

(a) Tentatively identified based on mass spectra comparison with NIST library;

(b) Comparison of RI with the RI of the genuine standard of the identified compound;

(c) Tentatively identified based on RI comparison using the Flavornet database (http://www.flavornet.org/).

Table 10 – Identification of the odorant compounds sensed in the extract obtained after acid hydrolysis of Freiminer grapes during GC–O analysis by correlation with the obtained GC–MS results.

RI GC–O	RI GC–MS	Compound	Odor descriptor	FD
1106.7	1109 [(c)]	*(+)–cis*–rose oxide [(c)]	Flowery	1
1184.7	1185 [(c)]	hexyl butanoate [(c)]	Fruity	1
1334.7	1335 [(c)]	benzyl butanoate [(c)]	Flowery	1
1646.8	1649 [(c)]	geranyl valerate [(c)]	Fruity, soap	1
1773.9	1773 [(c)]	10–epi–*γ*–eudesmol [(c)]	Fruity, sweet	1
2030.2	2024 [(c)]	*(E)*–isoeugenol [(c)]	Flowery, bittersweet	2
3116.3		unidentified	Fruity	1
3452.9		unidentified	Burnt	2

(a) Tentatively identified based on mass spectra comparison with NIST library;

(b) Comparison of RI with the RI of the genuine standard of the identified compound;

(c) Tentatively identified based on RI comparison using the Flavornet database (http://www.flavornet.org/).

In the three extracts of Freiminer grapes, several compounds were sensed, with a FD range from 1 to 16.

The odor compounds sensed on the non–polar extract of Freiminer grapes (Table 8) have low FD, from 1 to 4, being the compounds detected with the highest FD cuminol and oleyl alcohol (FD=4).

The odorant compounds perceived in the extract obtained after enzymatic hydrolysis of Freiminer grapes (Table 9) have a FD range from 1 to 16, being *(E)*–8–hydroxylinalool the only compound with FD=16.

Furthermore, on the extract obtained after acid hydrolysis of Freiminer grapes (Table 10), only compounds with a FD range from 1 to 2 were perceived, being *(E)*–isoeugenol the only compound with FD=2.

Regarding this information it is possible to say that, for Freiminer grapes, the major odor contributions come from their extract obtained after enzymatic hydrolysis, by action of *(E)*–8–hydroxylinalool (FD=16).

3.1.4 – Traminer

Table 11 – Identification of the odorant compounds sensed in the non–polar extract of Traminer grapes during GC–O analysis by correlation with the obtained GC–MS results.

RI GC–O	RI GC–MS	Compound	Odor descriptor	FD
783.8	789 [c]	1–hexenol [c]	Fruity, sweet	1
960.7	962 [c]	heptanol [c]	Grass, bittersweet	2
1031.0	1038.1	3,5–octadien–2–one [a]	Fruity, sweet	1
1085.4	1085.3	epoxylinalool [a]	Fruity, grass, sweet	2
1096.3	1096 [c]	3–nonenal [c]	Vegetable, ripped	1
1248.3	1250.6	1,1–dimethyl–2–propyl–cyclohexane [a]	Flowery, bittersweet	4
1264.7		unidentified	Flowery, bittersweet	8

(a) Tentatively identified based on mass spectra comparison with NIST library;

(b) Comparison of RI with the RI of the genuine standard of the identified compound;

(c) Tentatively identified based on RI comparison using the Flavornet database (http://www.flavornet.org/).

Table 12 – Identification of the odorant compounds sensed in the extract obtained after enzymatic hydrolysis of Traminer grapes during GC–O analysis by correlation with the obtained GC–MS results.

RI GC–O	RI GC–MS	Compound	Odor descriptor	FD
963.7	962 [c]	heptanol [c]	Grassy, bittersweet	1
987.7		unidentified	Pepper, burnt	1
999.7	1000 [c]	methyl hexanoate [c]	Fruity, sweet	1
1012.3	1017.61	2–ethyl hexanol [a] [b]	Fruity, sweet	1
1038.7	1038.1	3,5–octadien–2–one [a]	Fruity, sweet	1
1085.4	1087.7	epoxylinalool [a]	Fruity, sweet	1
1201.3		unidentified	Tomato, ripped, sweet	4
1238.4	1237.9	geranial [a]	Flowery, bittersweet	8
1426.7	1425 [c]	*(–)–γ*–elemene [c]	Flowery, bittersweet	4

(a) Tentatively identified based on mass spectra comparison with NIST library;

(b) Comparison of RI with the RI of the genuine standard of the identified compound;

(c) Tentatively identified based on RI comparison using the Flavornet database (http://www.flavornet.org/).

Table 13 – Identification of the odorant compounds sensed in the extract obtained after acid hydrolysis of Traminer grapes during GC–O analysis by correlation with the obtained GC–MS results.

RI GC–O	RI GC–MS	Compound	Odor descriptor	FD
1015.4	1013.0	*o*–cymene [b]	Fruity, pepper	1
1060.5	1062.6	myrcenol [a]	Flowery, sweet	1
1088.5	1092.0	*p*–menthen–9–al [a]	Fruity, sweet	4
1285.2	1284.3	geranic acid [a]	Fruity, sweet	1

(Table 13 – continued)

RI GC–O	RI GC–MS	Compound	Odor descriptor	FD
1293.4	1290.6	*β*–damascenone [(a) (b)]	Fruity	1
1442.7	1445 [(c)]	*β*–farnesene [(c)]	Fruity, sweet	4
1484.1		unidentified	Sweet	1
1635.0	1634.6	syringe aldehyde [(a)]	Flowery, sweet	4

(a) Tentatively identified based on mass spectra comparison with NIST library;

(b) Comparison of RI with the RI of the genuine standard of the identified compound;

(c) Tentatively identified based on RI comparison using the Flavornet database (http://www.flavornet.org/).

According to Table 11, the compounds sensed in the non–polar extract of Traminer grapes have a FD range from 1 to 8. However, the compound sensed at the highest FD (FD=8) was not identified. The perceived odor compounds have a FD range from 1 to 4, being 1,1–dimethyl–2–propyl– cyclohexane the only compound with FD=4.

The compounds perceived in the extract obtained after enzymatic hydrolysis of Traminer grapes (Table 12) have a FD range from 1 to 8, being the key odorants geranial (FD=8) and *(–)–γ*–elemene (FD=4).

As seen in Table 13, the compounds sensed in the extract obtained after acid hydrolysis of Traminer grapes have a FD range from 1 to 4. The compounds with FD=4 are *p*–menthen–9–al, *β*–farnesene and syringe aldehyde.

Regarding this information it is possible to say that, for Traminer grapes, the major detected odor contributions come from all the extracts, with 1,1–dimethyl–2–propyl–cyclohexane (FD=4) from the non–polar extract, geranial (FD=8) and *(–)–γ*–elemene (FD=4) from the extract obtained after enzymatic hydrolysis and *p*–menthen–9–al (FD=4), *β*–farnesene (FD=4) and syringe aldehyde (FD=4) from the extract obtained after acid hydrolysis of Traminer grapes.

3.1.5 – Jutrzenka

Table 14 – Identification of the odorant compounds sensed in the non–polar extract of Jutrzenka grapes during GC–O analysis by correlation with the obtained GC–MS results.

RI GC–O	RI GC–MS	Compound	Odor descriptor	FD
788.0	789 [(c)]	1–hexenol [(c)]	Fruity, grassy, sweet	1
918.8	915.0	cyclopentane, 1,2,3,4,5–pentamethyl– [(a)]	Burnt	1
969.7		unidentified	Burnt	1
1060.5	1062.5	phenylethyl alcohol [(a)]	Flowery, bittersweet	4
1082.3	1077.0	nerol oxide [(a)]	Fruity, grassy	2
1093.2	1090.7	epoxylinalool [(a)]	Fruity, grassy, sweet	2
1279.1	1277.1	pentanlactone [(a)]	Bittersweet	1
1409.2	1409 [(c)]	lauric aldehyde [(c)]	Flowery, bittersweet	4
1466.6	1462.9	cyclohexane, 1,2,4–trimethyl– [(a)]	Grassy	1
1484.1		unidentified	Grassy	1

(a) Tentatively identified based on mass spectra comparison with NIST library;

(b) Comparison of RI with the RI of the genuine standard of the identified compound;

(c) Tentatively identified based on RI comparison using the Flavornet database (http://www.flavornet.org/).

Table 15 – Identification of the odorant compounds sensed in the extract obtained after enzymatic hydrolysis of Jutrzenka grapes during GC–O analysis by correlation with the obtained GC–MS results.

RI GC–O	RI GC–MS	Compound	Odor descriptor	FD
939.7	938 [(c)]	2–ethylpyridine [(c)]	Flowery	1
1009.2	1010 [(c)]	ethyl lactate [(c)]	Sweet	1

(Table 15 – continued)

RI GC–O	RI GC–MS	Compound	Odor descriptor	FD
1059.0	1061.6	phenylethyl alcohol [(a)]	Flowery, bittersweet	2
1090.1	1090.2	epoxylinalool [(a)]	Fruity, sweet	2
1219.7	1220.8	neral [(a)]	Strange, bread–like	4
1272.9	1274.6	2,6–dimethyl–3,7–octadiene–2,6–diol [(a)]	Flowery, bittersweet	4
1439.5	1444.1	*trans*–2,7–dimethyl–3,6–octadien–2–ol [(a)]	Fruity, sweet	4

(a) Tentatively identified based on mass spectra comparison with NIST library;

(b) Comparison of RI with the RI of the genuine standard of the identified compound;

(c) Tentatively identified based on RI comparison using the Flavornet database (http://www.flavornet.org/).

Table 16 – Identification of the odorant compounds sensed in the extract obtained after acid hydrolysis of Jutrzenka grapes during GC–O analysis by correlation with the obtained GC–MS results.

RI GC–O	RI GC–MS	Compound	Odor descriptor	FD
933.8	938 [(c)]	2–ethylpyridine [(c)]	Flowery, bittersweet	1
1217.7	1202.1	camphene [(a)]	Flowery, sweet	1
1236.1	1242.0	*α*–terpinolene [(a)]	Flowery	1
1444.2	1445 [(c)]	*β*–farnesene [(c)]	Fruity, sweet	1
1643.4	1644.2	hexadecane [(a)]	Flowery	1
1655.0	1654 [(c)]	*β*–eudesmol [(c)]	Fruity, ripped	2

(a) Tentatively identified based on mass spectra comparison with NIST library;

(b) Comparison of RI with the RI of the genuine standard of the identified compound;

(c) Tentatively identified based on RI comparison using the Flavornet database (http://www.flavornet.org/).

According to Table 14, the detected odor compounds sensed in the non–polar extract of Jutrzenka grapes have a FD range from 1 to 4. The same happens with the detected odor compounds sensed in the extract obtained after enzymatic hydrolysis (Table 15). In the non–polar extract, the compounds with highest FD are phenylethyl alcohol (FD=4) and lauric aldehyde (FD=4), while in the extract obtained after enzymatic hydrolysis, the compounds with highest FD are neral (FD=4), 2,6–dimethyl–3,7–octadiene–2,6–diol (FD=4), and *trans*–2,7–dimethyl–3,6–octadien–2–ol (FD=4). The compounds perceived in the extract obtained after acid hydrolysis (Table 16) have a FD range from 1 to 2, being *β*–eudesmol the only compound with FD=2.

Regarding this information it is possible to say that, for Jutrzenka grapes, the major detected odor contributions come from the non–polar extract, with phenylethyl alcohol (FD=4) and lauric aldehyde (FD=4), and from the extract obtained after enzymatic hydrolysis, neral (FD=4), 2,6–dimethyl–3,7–octadiene–2,6–diol (FD=4), and *trans*–2,7–dimethyl–3,6–octadien–2–ol (FD=4).

From the six grape varieties that were studied, Jutrzenka was the only one that was previously analyzed. The main odorants discovered in wine produced from this variety were *β*–damascenone, 4–mercapto–4–methyl–2–pentanone, methional, 3–methyl butanolethyl decanoate, ethyl hexanoate, linalool and furaneol (14). None of these compounds were identified as key odorants in this Jutrzenka grapes analysis, which suggests the high impact of wine making process, or even the grape maturation state or appellation impact, among others.

3.1.6 – Adalmiina

Table 17 – Identification of the odorant compounds sensed in the non–polar extract of Adalmiina grapes during GC–O analysis by correlation with the obtained GC–MS results.

RI GC–O	RI GC–MS	Compound	Odor descriptor	FD
783.8	789 [(c)]	1–hexenol [(c)]	Fruity, grassy	8
1060.5	1063.0	phenylethyl alcohol [(a)]	Flowery, bittersweet	16

(Table 17 – continued)

RI GC–O	RI GC–MS	Compound	Odor descriptor	FD
1091.6	1098.2	betula [(a)]	Fruity, sweet	2
1297.5	1296.0	4–tetradecene [(a)]	Fruity, sweet	1

(a) Tentatively identified based on mass spectra comparison with NIST library;

(b) Comparison of RI with the RI of the genuine standard of the identified compound;

(c) Tentatively identified based on RI comparison using the Flavornet database (http://www.flavornet.org/).

Table 18 – Identification of the odorant compounds sensed in the extract obtained after enzymatic hydrolysis of Adalmiina grapes during GC–O analysis by correlation with the obtained GC–MS results.

RI GC–O	RI GC–MS	Compound	Odor descriptor	FD
788.0	789 [(c)]	1–hexenol [(c)]	Fruity, grassy	32
1077.6		unidentified	Flower, bittersweet, perfume	1
1401.2		unidentified	Flowery	1
1449.0		unidentified	Fruity, sweet	1
1652.0	1654.3	blumenol c [(a)]	Fruity, very sweet	1
1683.8	1682.7	dihydroactinidiolide [(a)]	Fruity, sweet	1

(a) Tentatively identified based on mass spectra comparison with NIST library;

(b) Comparison of RI with the RI of the genuine standard of the identified compound;

(c) Tentatively identified based on RI comparison using the Flavornet database (http://www.flavornet.org/).

Table 19 – Identification of the odorant compounds sensed in the extract obtained after acid hydrolysis of Adalmiina grapes during GC–O analysis by correlation with the obtained GC–MS results.

RI GC–O	RI GC–MS	Compound	Odor descriptor	FD
783.8	789 [(c)]	1–hexenol [(c)]	Fruity, grassy	1
1065.2		unidentified	Flowery, bittersweet	1
1094.8	1098.2	betula [(a)]	Fruity, sweet	1

(Table 19 – continued)

RI GC–O	RI GC–MS	Compound	Odor descriptor	FD
1417.2		unidentified	Flowery, bittersweet	1
1452.2	1454.7	1–(2,3,6–trimethylphenyl)–3–butene–2–one [(a)]	Flowery, bittersweet	8

(a) Tentatively identified based on mass spectra comparison with NIST library;

(b) Comparison of RI with the RI of the genuine standard of the identified compound;

(c) Tentatively identified based on RI comparison using the Flavornet database (http://www.flavornet.org/).

According to Table 17, the detected odor compounds sensed in the non–polar extract of Adalmiina grapes have a FD range from 1 to 16. The compound with highest FD are phenylethyl alcohol (FD=16) and 1–hexenol (FD=8).

The compounds sensed in the extract obtained after enzymatic hydrolysis (Table 18) have a FD range from 1 to 32, being 1–hexenol the only compound with FD=32.

The compounds perceived in the extract obtained after acid hydrolysis (Table 19) have a FD range from1 to 8, being 1–(2,3,6–trimethylphenyl)–3–butene–2–one the only compound with FD=8.

Regarding this information it is possible to say that, for Adalmiina grapes, the major detected odor contributions come from all three extracts, with 1–hexenol (FD=32) from the extract obtained after enzymatic hydrolysis (also being present with at FD=8 on the non–polar extract *), phenylethyl alcohol (FD=16) and 1–(2,3,6–trimethylphenyl)–3–butene–2–one (FD=8) from the extract obtained after acid hydrolysis of Adalmiina grapes.

A summary of the key odorants identified in each grape variety is described in Table 20.

Table 20 – Summary of the key odorants identified in each grape variety.

Grape variety	Identified key odorants	FD	Odor description
Mília	*β*–linalool	32	Fruity, sweet
	cis–*p*–menth–8–en–1–ol	32	Pepper, intense
	geranic acid	16	Fruity, sweet
	2,6–hexadienal	16	Fruity, ripped, sweet, intense
	isopulegol	8	Fruity, sweet
Merzling	*cis*–*p*–menth–8–en–1–ol	128	Fruity sweet
	4–terpineol	64	Sweet
	β–linalool	8	Pepper
Freiminer	*(E)*–8–hydroxylinalool	16	Flowery, bittersweet
Traminer	geranial	8	Flowery, bittersweet
	1,1–dimethyl–2–propyl– cyclohexane	4	Flowery, bittersweet
	(–)–*γ*–elemene	4	Flowery, bittersweet
	p–menthen–9–al	4	Fruity, sweet
	β–farnesene	4	Fruity, sweet
	syringe aldehyde	4	Flowery, sweet
Jutrzenka	phenylethyl alcohol	4	Flowery, bittersweet
	lauric aldehyde	4	Flowery, bittersweet
	neral	4	Strange, bread–like
	2,6–dimethyl–3,7–octadiene–2,6–diol	4	Flowery, bittersweet
	trans–2,7–dimethyl–3,6–octadien–2–ol	4	Fruity, sweet
Adalmiina	1–hexenol	32*	Flowery, bittersweet
	phenylethyl alcohol	16	Fruity, grassy
	1–(2,3,6–trimethylphenyl)–3–butene–2–one	8	Flowery, bittersweet

3.2 – Distribution of the sensed odorants – free and glycosidically–bound

Throughout the GC–O analysis of the odorant compounds, 145 sensations were recorded. Some compounds were present in more than one grape variety and some compounds were present, within the same variety, in different extracts.

It can be observed that in total, there were more sensed compounds in the glycosidically–bound form (55.6%) than on the free form (44.4%), even though the difference is not very discrepant. Such an result was expected, as there are evidences that glycosylated forms of odor compounds like terpenes and C_{13}–norisoprenoids are frequently more common than the free ones (8).

Figure 13 shows the percentage of the odor compounds detected in each of the grape varieties, in the free and glycosidically–bound form. Compounds both present, within the same grape variety, in the extract obtain after enzymatic and acid hydrolysis were only taken in consideration once.

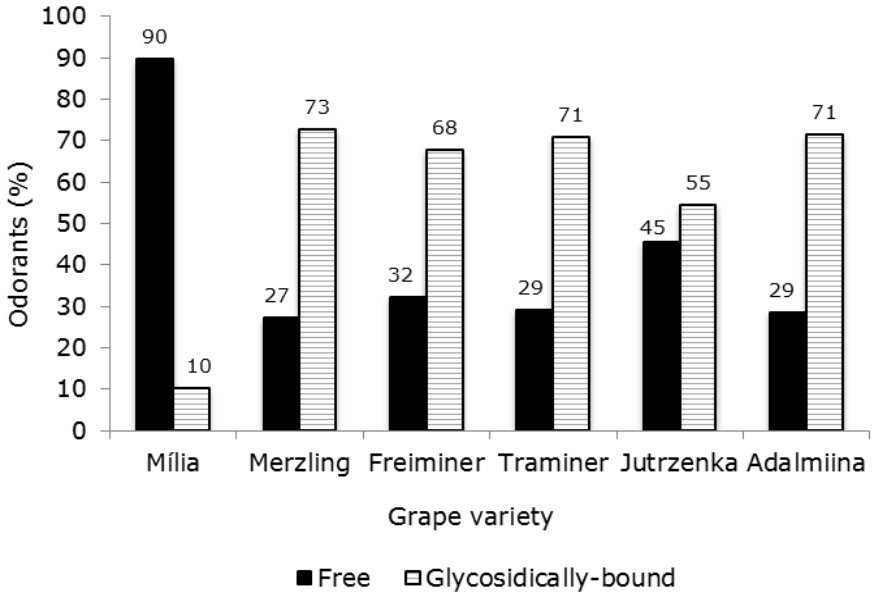

Figure 13 – Percentage of detected odors, during GC–O analysis, as free and glycosidically–bound compounds, in the six grape varieties.

It can be observed that only for Mília grapes there were a higher percentage of sensed free odor compounds (89.7%) than of sensed glycosidically–bound odor compounds

(10.3%), with a relatively big discrepancy. That fact and having in mind that no odors were detected in the extract obtained after enzymatic hydrolysis of Mília grapes, suggests that something could be wrong with that prepared sample. In the other grape varieties, the majority of the sensed odor compounds were in the glycosidically–bound form, as expected.

Figure 14 represents the key odor compound distribution between extracts.

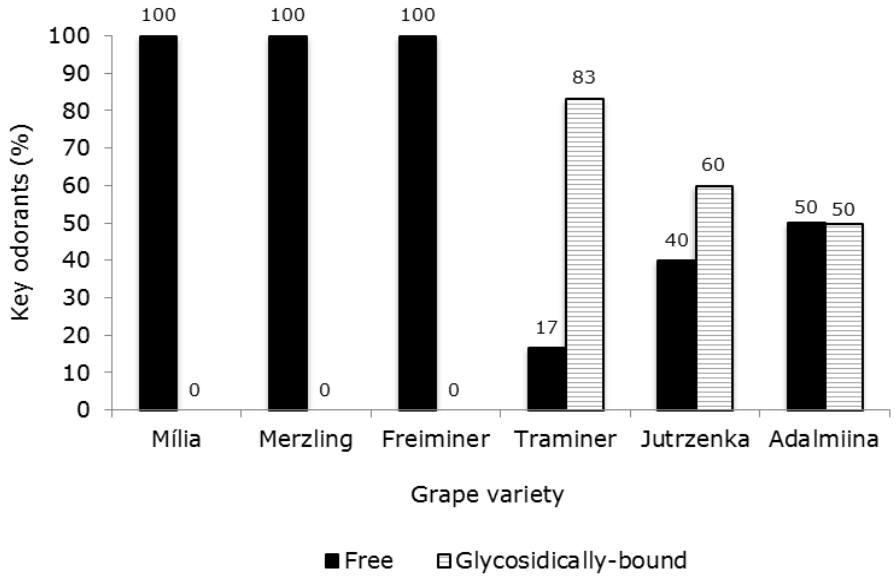

Figure 14 – Percentage of key odorants present in the free or glycosidically–bound form, in the six analysed grape varieties.

In Mília, Merzling and Freiminer grapes the key odorants were only sensed in the free from. However, in Traminer, Jutrzenka and Adalmiina grapes, the key odorants were sensed in both forms: in Traminer grapes 83% the key odorants are sensed in the glycosidically–bound form, as happens with Jutrzenka grapes at less extent, 60%. In Adalmiina grapes, the number of key odorants sensed in both fractions was equal.

Comparing Figures 13 and 14, it can be seen that despite the fact that Merzling, Freiminer, Traminer Jutrzenka and Adalmiina grapes have a higher percentage of overall odor compounds in the glycosidically–bound form, just Traminer and Jutrzenka grapes have a higher number of key odorant compounds present in the same form: if the glycosidically-bound odorants are more abundant that the free ones, it is probable that there are more key odorants in the glycosidically bound form. In Merzling and Freiminer

grapes there was not any key odorant detected in the glycosidically–bound form and in Adalmiina grapes the number of key odorants present in both forms was the same. Mília grapes were the only ones to have a higher percentage of overall odor compounds in the free form, and the same was verified with its number of key odorants, as already explained.

A summary of the key odorants present in free or glycosidically-bound form is represented on Table 21.

Table 21 – Summary of the key odorant compounds present as free or glycosidically-bound in the six analysed grape varieties.

	Free compounds	**Glycosidically–bound compounds sensed after enzymatic hydrolysis**	**Glycosidically–bound compounds sensed after acid hydrolysis**
Mília	β–linalool		
	cis–p–mentha–8–en–1–ol		
	geranic acid		
	2,6–hexadienal		
	isopulegol		
Merzling	cis–p–mentha–8–en–1–ol		
	4–terpineol		
	β–linalool		
Freiminer		(E)–8–hydroxylinalool	
Traminer	1,1–dimethyl–2–propyl–cyclohexane	geranial	p–menthen–9–al
		(–)–γ–elemene	β–farnesene
			syringe aldehyde
Jutrzenka	phenylethyl alcohol	neral	
	lauric aldehyde	2,6–dimethyl–3,7–octadiene–2,6–diol	
		trans–2,7–dimethyl–3,6–octadien–2–ol	
Adalmiina	phenylethyl alcohol	1–hexenol	1–(2,3,6–trimethylphenyl)–3–butene–2–one
	1–hexenol		

3.3 – Analysis of the odor description of the identified key odor compounds

Following is presented a comparison between the odorant compounds sensory descriptions noted during this analysis (Table 22) and the descriptions found in literature and/or in available databases.

Table 22 – Summary of the odor description of the identified key odorants in the six analysed grape varieties; N.I. = not identified.

Grape variety	Identified key odorants	Odor descriptor	Odor descriptor (literature)
Mília	β–linalool	Fruity, sweet	Fruity, flowery, lavender, orange, bergamot
	cis–*p*–mentha–8–en–1–ol	Pepper, intense	Musty
	geranic acid	Fruity, sweet	Green
	2,6–hexadienal	Fruity, ripped, sweet, intense	Green, fruity
	isopulegol	Fruity, sweet	N.I.
Merzling	*β*–linalool	Fruity sweet	Fruity, flowery, lavender, orange, bergamot
	4–terpineol	Sweet	Floral, musty
	cis–*p*–mentha–8–en–1–ol	Pepper	Musty
Freiminer	(*E)*–8–hydroxylinalool	Flowery, bittersweet	N.I.
Traminer	1,1–dimethyl–2–propyl–cyclohexane	Flowery, bittersweet	N.I.
	geranial	Flowery, bittersweet	Lemon, minty
	(–)–*γ*–elemene	Flowery, bittersweet	Green, woody
	p–menthen–9–al	Fruity, sweet	Spicy, herbal
	β–farnesene	Fruity, sweet	Citrus, sweet
	syringe aldehyde	Flowery, sweet	Green

(Table 22 – continued)

Grape variety	Identified key odorants	Odor descriptor	Odor descriptor (literature)
Jutrzenka	phenylethyl alcohol	Flowery, bittersweet	Flowery, rose, lilac, sweet
	lauric aldehyde	Flowery, bittersweet	Lily, citrus
	neral	Strange, bread–like	Lemon
	2,6–dimethyl–3,7–octadiene–2,6–diol	Flowery, bittersweet	N.I.
	trans–2,7–dimethyl–3,6–octadien–2–ol	Fruity, sweet	N.I.
Adalmiina	phenylethyl alcohol	Flowery, bittersweet	Flowery, rose, lilac, sweet
	1–hexenol	Fruity, grassy	Green
	1–(2,3,6–trimethylphenyl)–3–butene–2–one	Flowery, bittersweet	N.I.

According to the literature and to the Flavornet database: *β*–linalool has a fruity (14), flowery (7) (14) (32) (43), lavender (43), orange (7) and bergamot (42) smell, comparable with the fruity, sweet odor detected; 2,4–hexadienal has a green (42) (43), fruity (43) odor, likely similar to the fruity, ripped, sweet odor detected; geranic acid odor was described as a green odor (44), resembling the fruity, sweet odor detected; 4–terpineol has also a floral (7) (45), musty odor (42), probably with similarities with the sweet detected odor; geranial has a lemon, minty odor (42) probably resembling the bitterness of the flowery, bittersweet odor detected; *(–)*–*γ*–elemene has a green, woody odor (42) and a flowery, bittersweet odor was detected; *p*-menthen-9-al which as a spicy herbal odor (46), and a fruity sweet odor was detected; *β*–farnesene has a citrus, sweet odor (42) with similarities with the fruity, sweet odor detected; syringe aldehyde odor was described as green, and it was detected a flowery, sweet aroma (47); phenylethyl alcohol has a flowery (14) (30), rose (7) (30) (42), lilac (42), sweet (14) odor and a flowery, bittersweet odor was detected; lauric aldehyde has a lily, citrus odor (42) and a flowery, bittersweet odor was detected; 1–hexenol has a green odor (42) similar to the grassy, fruity odor detected.

An odor description to *(E)*–8–hydroxylinalool, 1,1–dimethyl–2–propyl–cyclohexane, 2,6–dimethyl–3,7–octadiene–2,6–diol, *trans*–2,7–dimethyl–3,6–octadien–2–ol, and 1–(2,3,6–trimethylphenyl)–3–butene–2–one was not found.

The sensory perceptions that did not quite match the ones found in the literature were *cis*–*p*–menth–8–en–1–ol, which has a musty odor (43), instead of the intense pepper odor detected; and neral has a lemon like odor (42), and a strange bread-like odor was detected.

It should be noted that the sensation described as "pepper" (on *cis*–*p*–menth–8–en–1–ol) was very clear, during GC–O analysis. That odor is normally associated with the presence of methoxypyrazines (8). However, no methoxypyrazines were identified. Regarding the strange bread-like odor sensed during the elution of neral, it is possible that some artefacts were formed during the GC-O analysis.

It is also important to remark that the GC-O analysis should be done by a group of people experienced with the approach, and that was not the case. So, it is possible that the description of some odors was imprecise.

In general, the identified aroma compounds of the six grape varieties analysed contributed to green, grassy, flowery, fruity, sweet or bittersweet odors, matching the overall description for grape aroma existent in the literature and on available databases.

3.4 – Dilution factors assessment

The general range of FD values (1–128) from which the odor compounds were detected was quite low, comparing for example, with the ones obtained in a previous study of Jutrzenka liquer wine (2–4096) (14). Furthermore, most of the identified odorants don't contribute significantly for the overall grape aroma (Figure 15). From 145 sensed odor compounds during GC–O analysis, more than 60% were only sensed in FD=1, little over 10% were sensed in FD=2 and FD=4, and less than 10% were sensed at a higher FD.

The FD values are indicators of the influence of the compound on the overall aroma of a given product. However one has to bear in mind that it was used a very non–standard detector (nose), and that several factors will influence the analysis: the nose response to odors can vary for different odorants; perception of aroma is highly dependent on the

person performing analysis, therefore results from one person will not supply the full picture of the product; GC–O should be performed by people with long experience in this type of analyses; the description of particular aromas can differ between people and can be imprecise (fruity, but not specifying particular fruit, or flowery, but not specifying a special flower name); FD values will be also highly dependable on the extraction and especially concentration of extract. Therefore for the same fruit, the results can be very different. It shows well the proportions of flavor compounds in the given sample, but it cannot be used directly to compare different samples (in this case grape varieties).

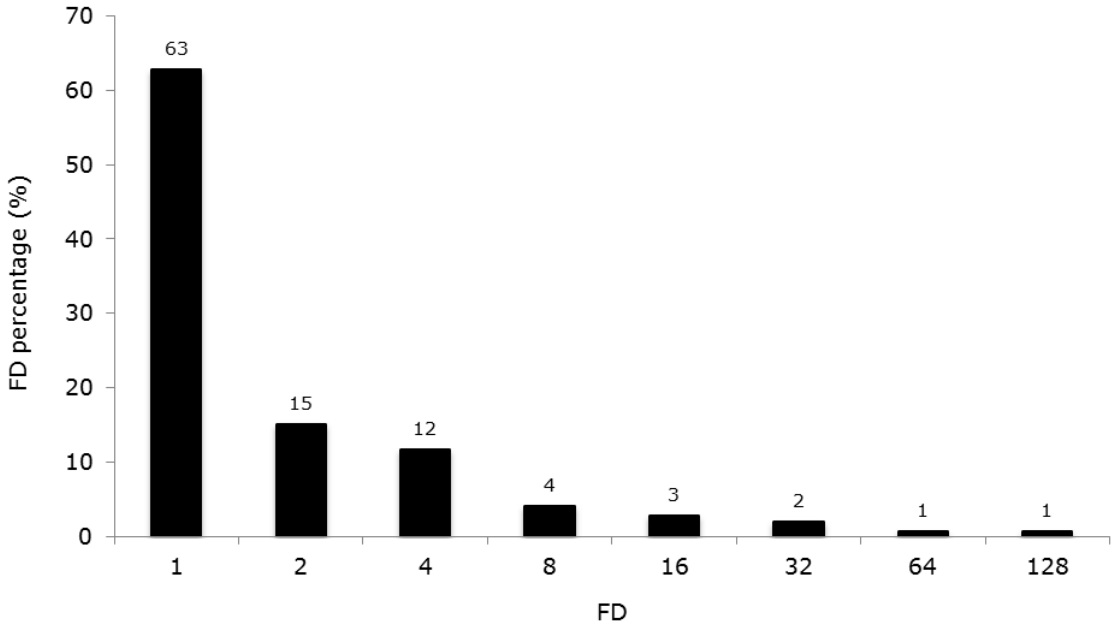

Figure 15 – FD percentage in which odorant compounds were perceived.

To overcome the difficulties related to it, FD is usually only a first step in finding compounds responsible for aroma of a given product. Then they can be identified by GC–MS and quantified, and the OAV's can be applied. This approach would relate the concentration of analysed compounds to their sensory importance.

3.5 – Overall key–odorant compound relevance in general grapes

On Table 20 are listed the key odorant compounds sensed during the analysis of 6 grape varieties.

It is now interesting to investigate whether the key odor compounds identified are commonly sensed or present in other grape varieties.

β–linalool was identified, as key odorant, in Jutrzenka liquer wine (14), *Vitis vinifera* L. cv. Fiano grapes (7) and in *Vitis vinifera* L. cv. Melon B (45). The same compound was also identified, however not as a key odorant, in Aglianico, Uva di Troia (31), Albariño (41), Bual and Bastardo (48) grapes. In *Vitis vinifera* L. cv. Fiano grapes (7) it was found in a glycosidically–bound form, being identified only after enzymatic hydrolysis. However, in this analysis, *β*–linalool was only odor relevant in the free form.

4–terpineol was identified, as key odorant, in *Vitis vinifera* L. cv. Fiano grapes (7) and *Vitis vinifera* L. cv. Melon B (45). It was also identified in Jutrzenka liquer wine (14) and in Albariño grapes (41), but not as a main odorant. In *Vitis vinifera* L. cv. Fiano grapes (7), 4–terpineol was identified as a main odorant occurring after acid hydrolysis. However, in this analysis, 4–terpineol was only odor relevant in the free form.

Phenylethyl alcohol was identified, as a key odorant, in Jutrzenka liquer wine (41) and in *Vitis vinifera* L. cv. Fiano grapes (7), having also being detected, although without information about odorant relevance, in Aglianico, Uva di Troia (31), Albariño (41), Bual and Bastardo (48) grapes. In *Vitis vinifera* L. cv. Fiano grapes (7), phenylethyl alcohol was identified as a main odorant occurring after enzymatic hydrolysis. However, in this analysis, it was only odor relevant in the free form.

Although not having been identified as key odorants, geranic acid was identified in Jutrzenka liquer wine (14), in *Vitis vinifera* L. cv. Fiano grapes (7) and in the skin of Bastardo grapes (48); (E)–8–hydroxylinalool was identified in *Vitis vinifera* L. cv. Melon B (45); syringe aldehyde was identified in in Aglianico, Uva di Troia grapes (31); 2,6–dimethyl–3,7–octadiene–2,6–diol was identified in Jutrzenka liquer wine (14); p-mentha-8-en-1-ol was found in the pulp and skin of Bual and Bastardo grapes (48) and 1–hexenol was identified in *Vitis vinifera* L. cv. Fiano grapes (7) and on the pulp and skin of Bual and Bastardo grapes (48). Neral and geranial have been identified in Bimeijia grapes (49) and

in *Vitis vinifera* L. cv. Scheurebe (50), despite having a low occurrence. Furthermore, neral was also indentified in the pulp and skin of Bual and Bastardo grapes (48).

4 – Conclusion

This study presents results from the olfactometric experiment performed in order to identify the key odorant compounds of six grape varieties: Mília, Merzling, Freiminer, Traminer, Jutrzenka and Adalmiina.

The most relevant odor components identified for Mília grapes were *β*–linalool (FD=32), *cis*–*p*–menth–8–en–1–ol (FD=32), geranic acid (FD=16), 2,6–hexadienal (FD=16) and isopulegol (FD=8) as free flavor compounds. For Merzling grapes, the identified key odorants were *cis*–*p*–menth–8–en–1–ol (FD=128), 4–terpineol (FD=64) and *β*–linalool (FD=8), also as free flavor compounds. For Freiminer grapes, *(E)*–8–hydroxylinalool (FD=16) was the key odor compound identified, as glycosidically–bound flavor compound. For Traminer grapes, the key aroma compound present in a free form was 1,1–dimethyl–2–propyl–cyclohexane (FD=4), and the glycosidically–bound key aroma compounds detected were geranial (FD=8), *(–)*–*γ*–elemene (FD=4), *p*–menthen–9–al (FD=4), *β*–farnesene (FD=4) and syringe aldehyde (FD=4). For Jutrzenka grapes, phenylethyl alcohol (FD=4) and lauric aldehyde (FD=4) were the major aroma compounds present in their free form, and neral (FD=4), 2,6–dimethyl–3,7–octadiene–2,6–diol (FD=4) and *trans*–2,7–dimethyl–3,6–octadien–2–ol (FD=4) were the glycosidically–bound key odor compounds detected. Finally, for Adalmiina grapes, the free key aroma compounds identified were phenylethyl alcohol (FD=16) and 1–hexenol (FD=8), and the glycosidically–bound key aroma compounds detected were 1–hexenol (FD=32) and 1–(2,3,6–trimethylphenyl)–3–butene–2–one (FD=8).

With this information in mind, it is possible to say that a specific key odorant pattern was established for each one of the six grape varieties under study.

All six grape varieties had more sensed odorants in the glycosidically-bound form except Mília grapes. However, only Traminer and Jutrzenka grapes had more key odorants in the glycosidically-bound form. Mília, Merzling and Traminer grapes only had key odorants in the free form and Adalmiina grapes had equal number of key odorants in both forms.

In general, the compounds of the six grape varieties analysed had a flowery, grassy, green, fruity, bittersweet or sweet aroma, matching the general description of grape aroma, found in the literature and on available databases.

This analysis revealed the presence of some odorants, some of which are well known grape flavor components. However, most of the identified odorants don't contribute significantly for the overall grape aroma. It also was noted the absence of some odor compounds normally present in grapes.

The general range of FD values (1–128) from which the odor compounds were detected was quite low, but it is important to remember that there are several factors that could have influence the analysis: inexperience with the GC–O technique, different odor responses for the same compound, uneven extraction techniques. Having that in mind, the actual ability of some of these odorants to influence grape aroma will have to be confirmed by further experiments.

In future work,

- The sample preparation and extraction technique should be perfected, in order to be able to detect odors in samples with higher FD's;
- Comprehensive two–dimensional gas chromatography (GCxGC) could be applied in order to identify the compounds that remained without identification and to confirm the identifications already done;
- It would be interesting to submit these grape varieties through an experienced panel of professionals, capable of distinguishing particular odor notes present on the grapes;
- It would also be interesting to analyze the change in the odor profile in wines produced using these grapes.

5 – References

1. Ruberto G, Renda A, Amico V, Tringali C. Volatile components of grape pomaces from different cultivars of Sicilian *Vitis vinifera* L. Bioresource Technology. 2008;99(2):260-268.
2. Handbook of Fruit and Vegetable Flavors. Hui YH, editor: Wiley; 2010.
3. Martín-Belloso O, Marsellés-Fontanet AR. Grape Juice. In: Hui YH, editor. Handbook of Fruits and Fruit Processing. 1 ed: Blackwell Publishing; 2006.
4. Jacobson JL. Introduction to Wine Laboratory Practices and Procedures: Springer; 2006.
5. Prosen H, Janes L, Strlic M, Rusjan D, Kocar D. Analysis of free and bound aroma compounds in grape berries using headspace solid-phase microextraction with GC-MS and a preliminary study of solid-phase extraction with LC-MS. Acta Chimica Slovenica. 2007;54(1):25-32.
6. Roubelakis-Angelakis KA, editor. Grapevine Molecular Physiology and Biotechnology. 2 ed: Springer; 2009.
7. Ugliano M, Moio L. Free and hydrolytically released volatile compounds of *Vitis vinifera* L. cv. Fiano grapes as odour-active constituents of Fiano wine. Analytica Chimica Acta. 2008;621(1):79-85.
8. Ribéreau-Gayon P, Glories Y, Maujean A, Dubourdieu D. Handbook of Enology. 2 ed: Wiley; 2006.
9. Ham JE, Proper SP, Wells JR. Gas-phase chemistry of citronellol with ozone and OH radical: Rate constants and products. Atmospheric Environment. 2006;40(4):726-735.
10. Letizia CS, Cocchiara J, Lalko J, Api AM. Fragrance material review on linalool. Food and Chemical Toxicology. 2003;41(7):943-964.
11. Puglisi CJ, Elsey GM, Prager RH, Skouroumounis GK, Sefton MA. Identification of a precursor to naturally occurring beta-damascenone. Tetrahedron Letters. 2001;42(39):6937-6939.
12. Lalko J, Lapczynski A, McGinty D, Bhatia S, Letizia CS, Api AM. Fragrance material review on beta-ionone. Food and Chemical Toxicology. 2007;45:S241-S247.

13. Koch A, Doyle CL, Matthews MA, Williams LE, Ebeler SE. 2-Methoxy-3-isobutylpyrazine in grape berries and its dependence on genotype. Phytochemistry. 2010;71(17-18):2190-2198.
14. Jelen HH, Majcher M, Dziadas M, Zawirska-Wojtasiak R, Czaczyk K, Wasowicz E. Volatile compounds responsible for aroma of Jutrzenka liquer wine. Journal of Chromatography A. 2011;1218(42):7566-7573.
15. Tarko T, Duda-Chodak A, Sroka P, Satora P, Jurasz E. Physicochemical and antioxidant properties of selected polish grape and fruit wines. ACta Scientiarum Polonorum. 2008;7:35-45.
16. Golesz V. Polecane odmiany winorośli. Vineyard Golesz; 2013 [cited 2013 4/5/2013]; Available from: http://www.winnica.golesz.pl/odmiany-winorosli.html#winab.
17. Carolyn Fisher TRS. Food Flavours, Biology and Chemistry: RSC Paperbacks; 1997.
18. Mathilde Charles PP, Florence Texier, Gaelle Arvisenet, Evelyne Vigneay, Emira Mehinagic, Carole Prost. The 'Mouth to Nose Merging System': A novel approach to study the impact of odour on other sensory perceptions. Food Quality and Preference. 2013(28):264 - 270.
19. Jelen H, Majcher M, Dziadas M. Sample Preparation for Food Analysis (Flavors/Off-Flavors). Compreensive Sampling and Sample Preparation: Elsevier; 2012.
20. Guth H, Rusu M. Food matrices - determination of odorant partition coefficients and application of models for their prediction. Food Chemistry. 2008;108(4):1208-1216.
21. Belitz HD, Grosch W, Schieberle P. Food Chemistry. 4th ed: Springer; 2009.
22. Komthong P, Hayakawa S, Katoh T, Igura N, Shimoda M. Determination of potent odorants in apple by headspace gas dilution analysis. Lwt-Food Science and Technology. 2006;39(5):472-478.
23. Zellner BD, Dugo P, Dugo G, Mondello L. Gas chromatography-olfactometry in food flavour analysis. Journal of Chromatography A. 2008;1186(1-2):123-143.

24. Atanasova B, Thomas-Danguin T, Langlois D, Nicklaus S, Chabanet C, Etievant P. Perception of wine fruity and woody notes: influence of peri-threshold odorants. Food Quality and Preference. 2005;16(6):504-510.
25. Jackson RS. Wine Science, Principles and Applications. 3 ed: Academic Press / Elsevier; 2008.
26. Marsili R, editor. Flavor, Fragrance and Odor Analysis: Marcel Dekker, Inc.; 2002.
27. Augusto F, Lopes ALE, Zini CA. Sampling and sample preparation for analysis of aromas and fragrances. Trac-Trends in Analytical Chemistry. 2003;22(3):160-169.
28. Plutowska B, Wardencki W. Application of gas chromatography-olfactometry (GC-O) in analysis and quality assessment of alcoholic beverages - A review. Food Chemistry. 2008;107(1):449-463.
29. Zwir-Ferenc A, Biziuk M. Solid phase extraction technique - Trends, opportunities and applications. Polish Journal of Environmental Studies. 2006;15(5):677-690.
30. Sánchez-Palomo E, García-Carpintero EG, Gallego MÁG, Viñas MÁG. The Aroma of Rojal Red Wines from La Mancha Region – Determination of Key Odorants In: Salih DB, editor. Gas Chromatography in Plant Science, Wine Technology, Toxicology and Some Specific Applications InTech; 2012. p. 147-170.
31. Genovese A, Lamorte SA, Gambuti A, Moio L. Aroma of Aglianico and Uva di Troia grapes by aromatic series. Food Research International. 2013;53(1):15-23.
32. Canuti V, Conversano M, Calzi ML, Heymann H, Matthews MA, Ebeler SE. Headspace solid-phase microextraction-gas chromatography-mass spectrometry for profiling free volatile compounds in Cabernet Sauvignon grapes and wines. Journal of Chromatography A. 2009;1216(15):3012-3022.
33. Cuevas-Glory LF, Pino JA, Santiago LS, Sauri-Duch E. A review of volatile analytical methods for determining the botanical origin of honey. Food Chemistry. 2007;103(3):1032-1043.
34. Jiang W, Fan W, Xu Y, Zhao Ga, Li J, Yu Y. Analysis of free terpenoids in four *Vitis vinifera* varieties using solvent assisted flavour evaporation and gas

chromatography-tadem mass spectrometry. Se pu = Chinese journal of chromatography / Zhongguo hua xue hui. 2007;25(6):881-886.

35. van Ruth SM. Methods for gas chromatography-olfactometry: a review. Biomolecular Engineering. 2001;17(4-5):121-128.
36. Hassan M. Gas Chromatography and Mass Spectrometry (GC-MS). Institute of Biotechnology, GC UniversityLahore, 2011.
37. Settle F, editor. Handbook of Instrumental Techniques for Analytical Chemistry: Prentice Hall; 1997.
38. Careri M, Bianchi F, Corradini C. Recent advances in the application of mass spectrometry in food-related analysis. Journal of Chromatography A. 2002;970(1-2):3-64.
39. Kondjoyan N, Berdagué J-L. A compilation of relative retention indices for the analysis of aromatic compounds. Flaveur L, editor1996.
40. Zhang J, Fang A, Wang B, Kim SH, Bogdanov B, Zhou Z, et al. iMatch: A retention index tool for analysis of gas chromatography-mass spectrometry data. Journal of Chromatography A. 2011;1218(37):6522-6530.
41. Diéguez SC, Lois LaC, Gómez EF, de la Peña MLG. Aromatic composition of the *Vitis vinifera* grape Albariño. LWT - Food Science and Technology. 2003;36(6):585-590.
42. Acree T, Arn H. Flavornet. [cited 2013]; Available from: http://www.flavornet.org/index.html.
43. Mahattanatawee K, Goodner K, Baldwin E. Volatile constituents and character impact compounds of selected Florida's tropical fruit. Proceedings of the Florida State Horticultural Society. 2005(118):414-418.
44. geranic acid. Perfumer & Flavorist magazine; [cited 2013]; Available from: http://www.thegoodscentscompany.com/data/rw1001951.html.
45. Schneider R, Razungles A, Augier C, Baumes R. Monoterpenic and norisoprenoidic glycoconjugates of *Vitis vinifera* L. cv. Melon B. as precursors of odorants in Muscadet wines. Journal of Chromatography A. 2001;936(1-2):145-157.
46. para−menth−1−en−9−al. Perfumer & Flavorist magazine; Available from: http://www.thegoodscentscompany.com/data/rw1035371.html.

47. syringaldehyde. Perfumer & Flavorist magazine; [cited 2013]; Available from: http://www.thegoodscentscompany.com/data/rw1026801.html.
48. Perestrelo R, Barros AS, Rocha SM, Câmara JS. Optimisation of solid-phase microextraction combined with gas chromatography–mass spectrometry based methodology to establish the global volatile signature in pulp and skin of *Vitis vinifera* L. grape varieties. Talanta. 2011;85(3):1483-1493.
49. Yang C, Wang Y, Wu B, Fang J, Li S. Volatile compounds evolution of three table grapes with different flavour during and after maturation. Food Chemistry. 2011;128(4):823-830.
50. Luan F, Mosandl A, Münch A, Wüst M. Metabolism of geraniol in grape berry mesocarp of *Vitis vinifera* L. cv. Scheurebe: demonstration of stereoselective reduction, E/Z-isomerization, oxidation and glycosylation. Phytochemistry. 2005;66(3):295-303.

Appendixes – GC-MS chromatograms of the grape extracts from all analysed grape varieties

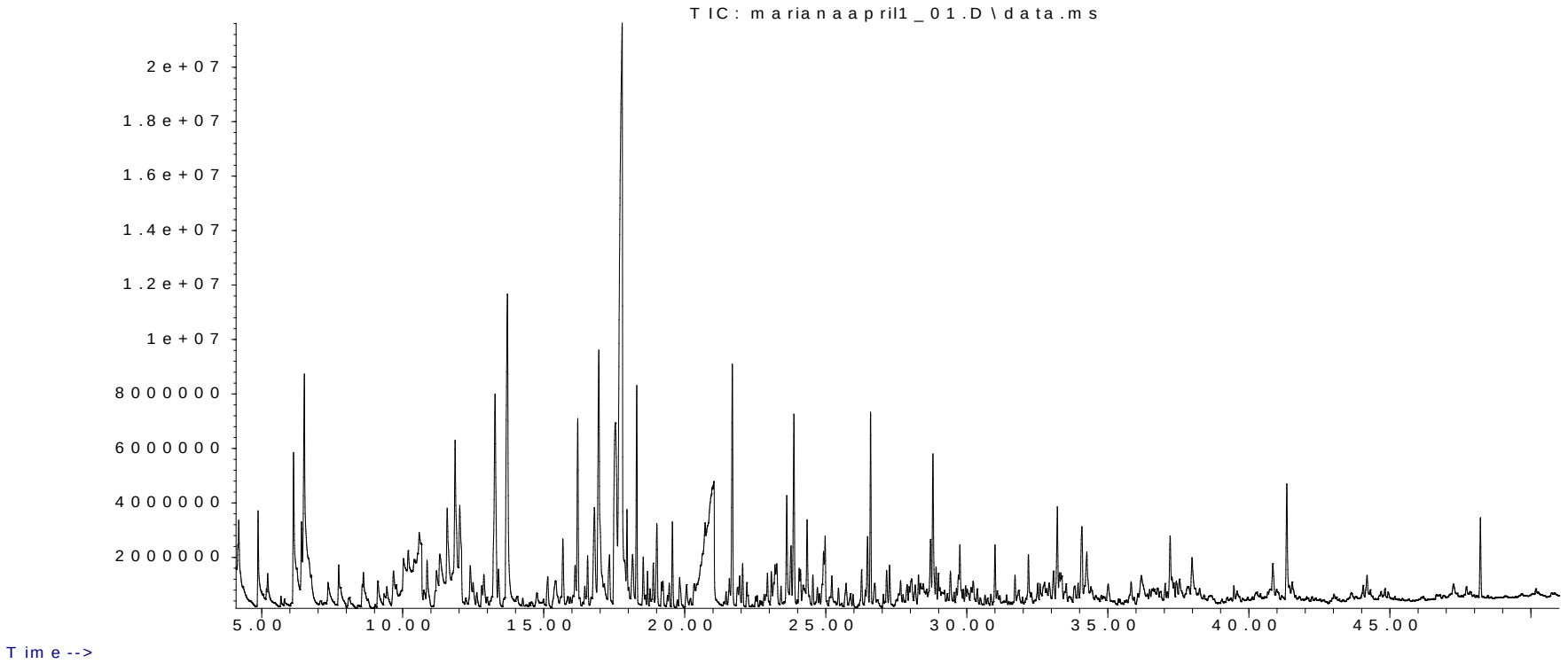

Figure 16 – GC-MS chromatogram of Mília grapes non–polar extract.

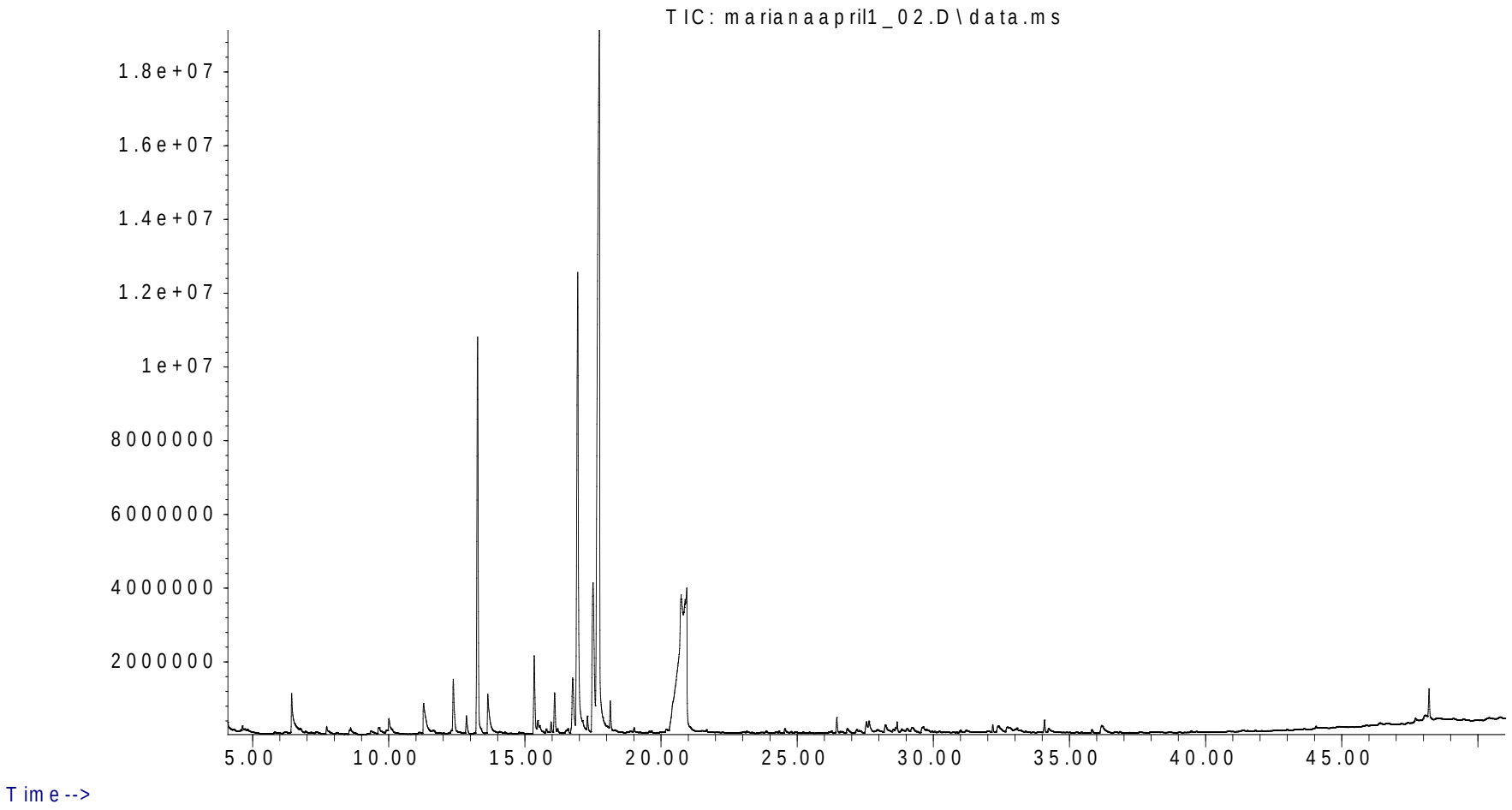

Figure 17 – GC-MS chromatogram of Mília grapes extract after enzymatic hydrolysis.

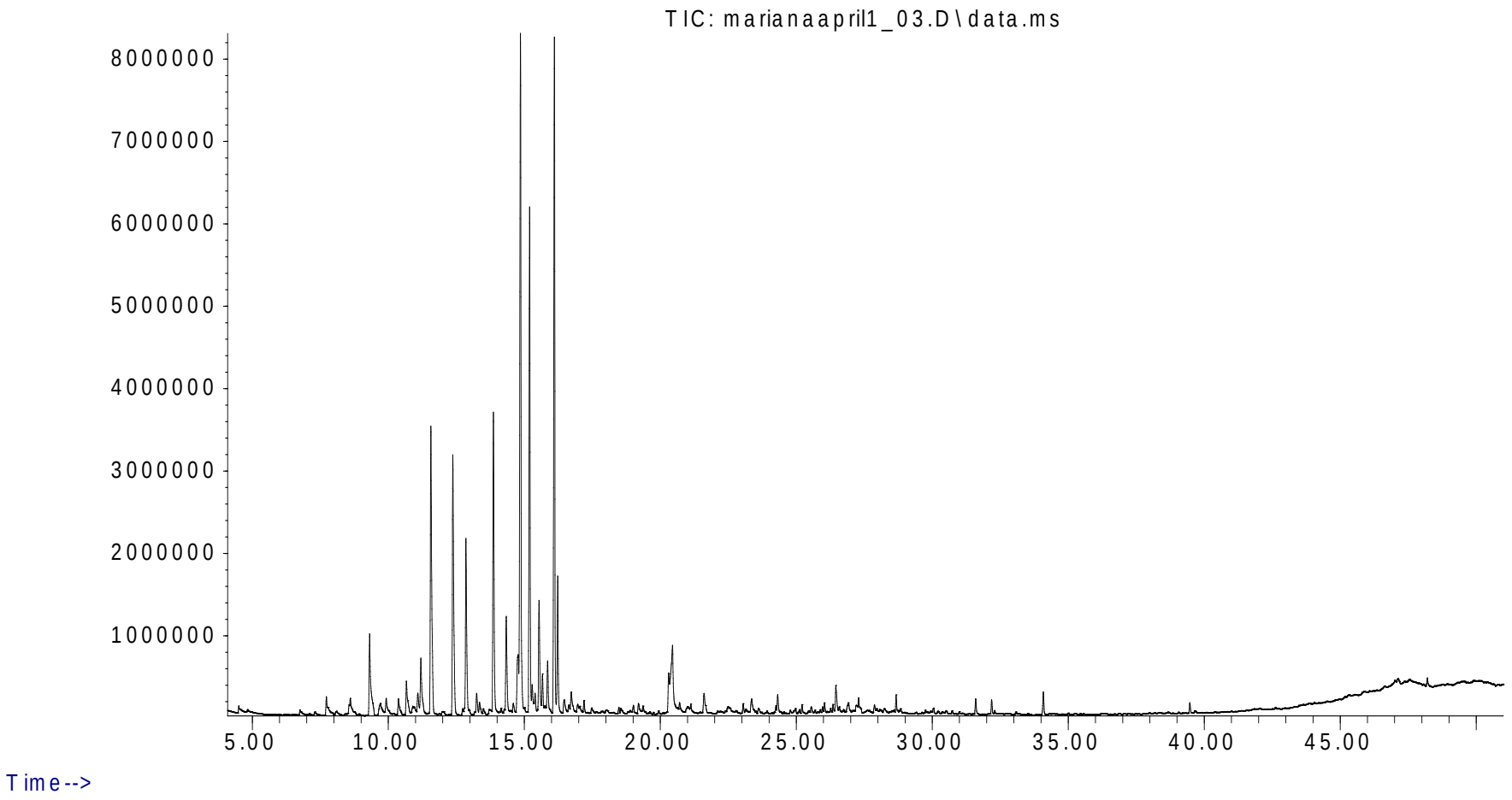

Figure 18 – GC-MS chromatogram of Mília grapes extract after acid hydrolysis.

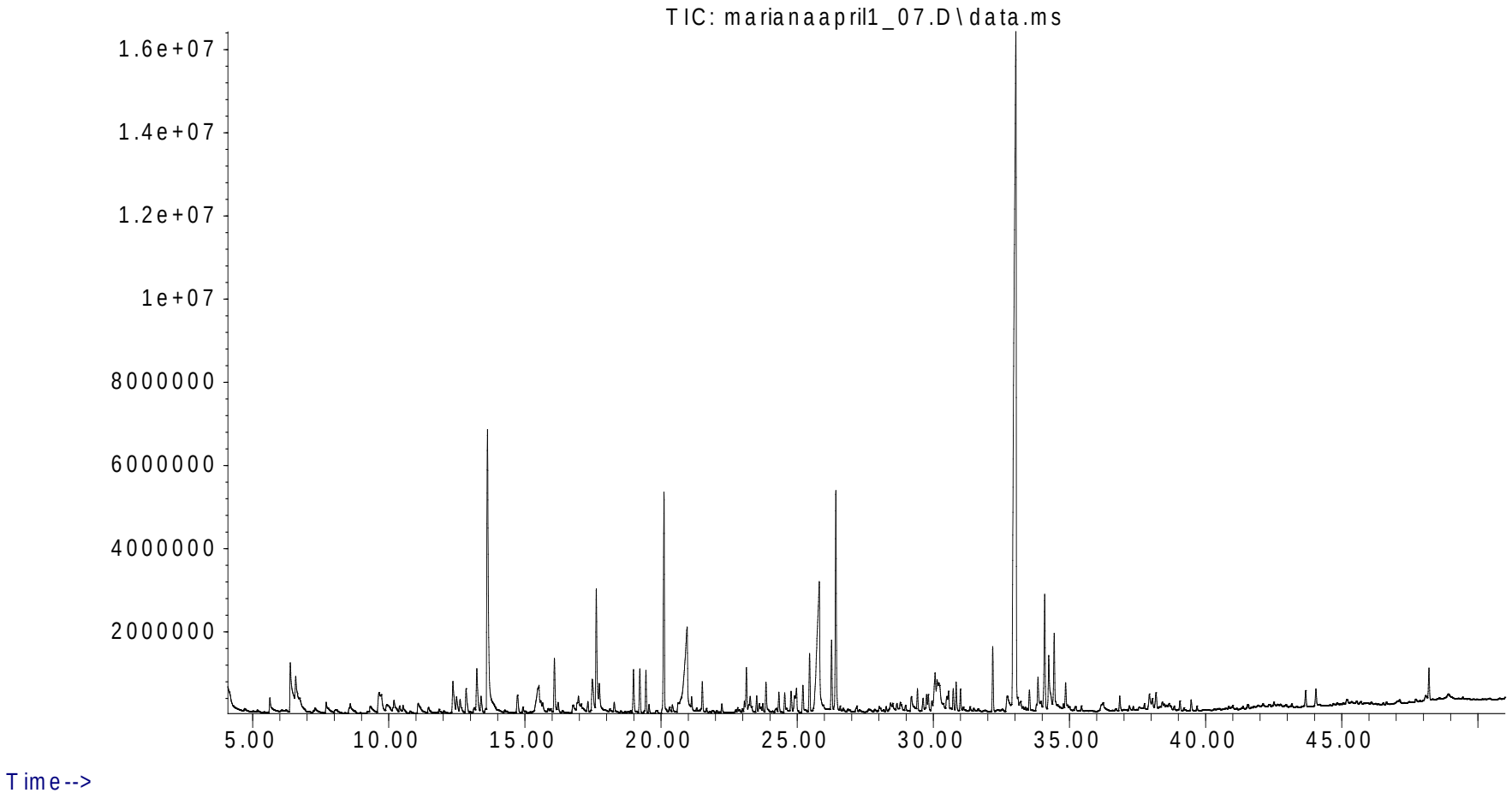

Figure 19 – GC-MS chromatogram of Merzling grapes non–polar extract.

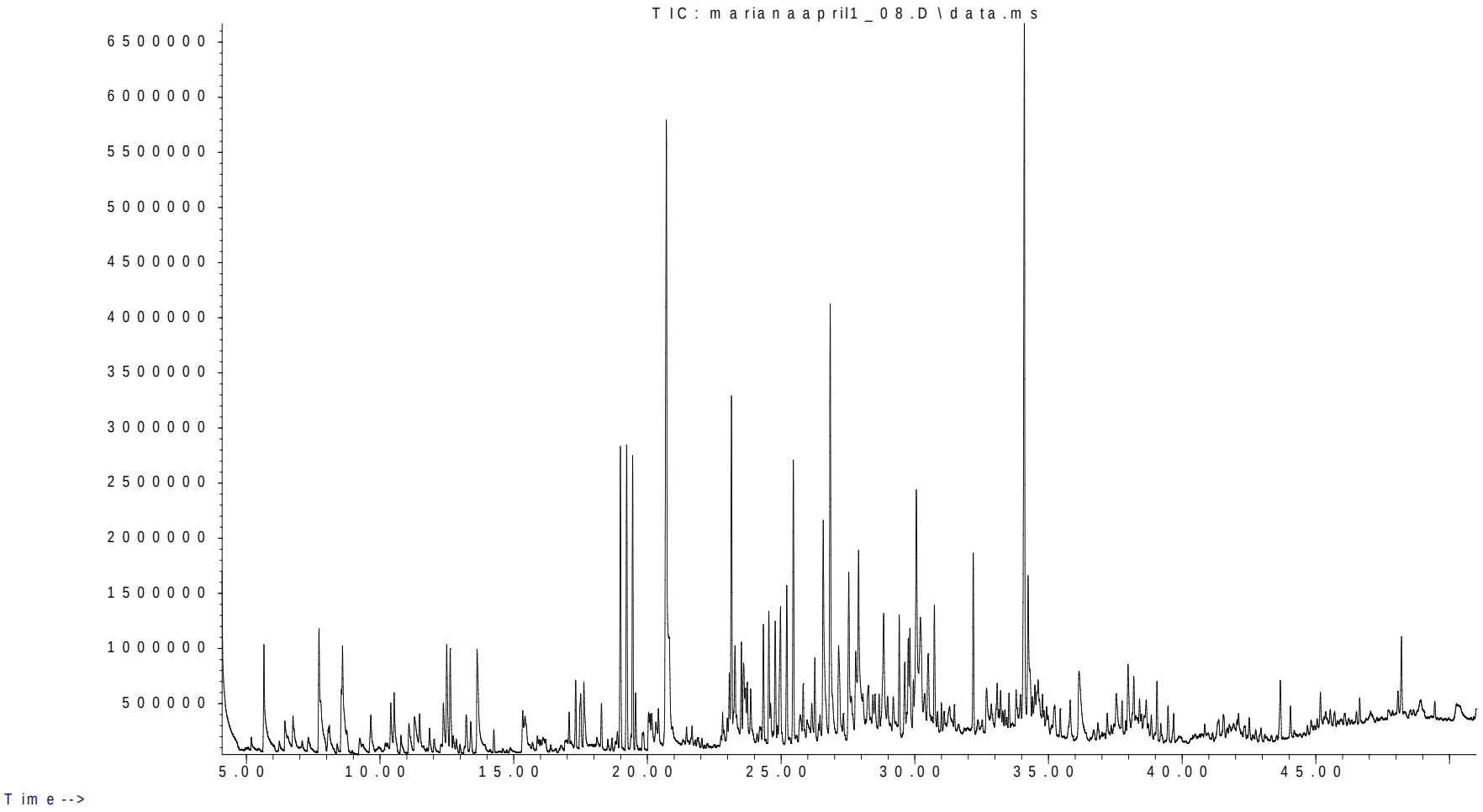

Figure 20 – GC-MS chromatogram of Merzling grapes extract after enzymatic hydrolysis.

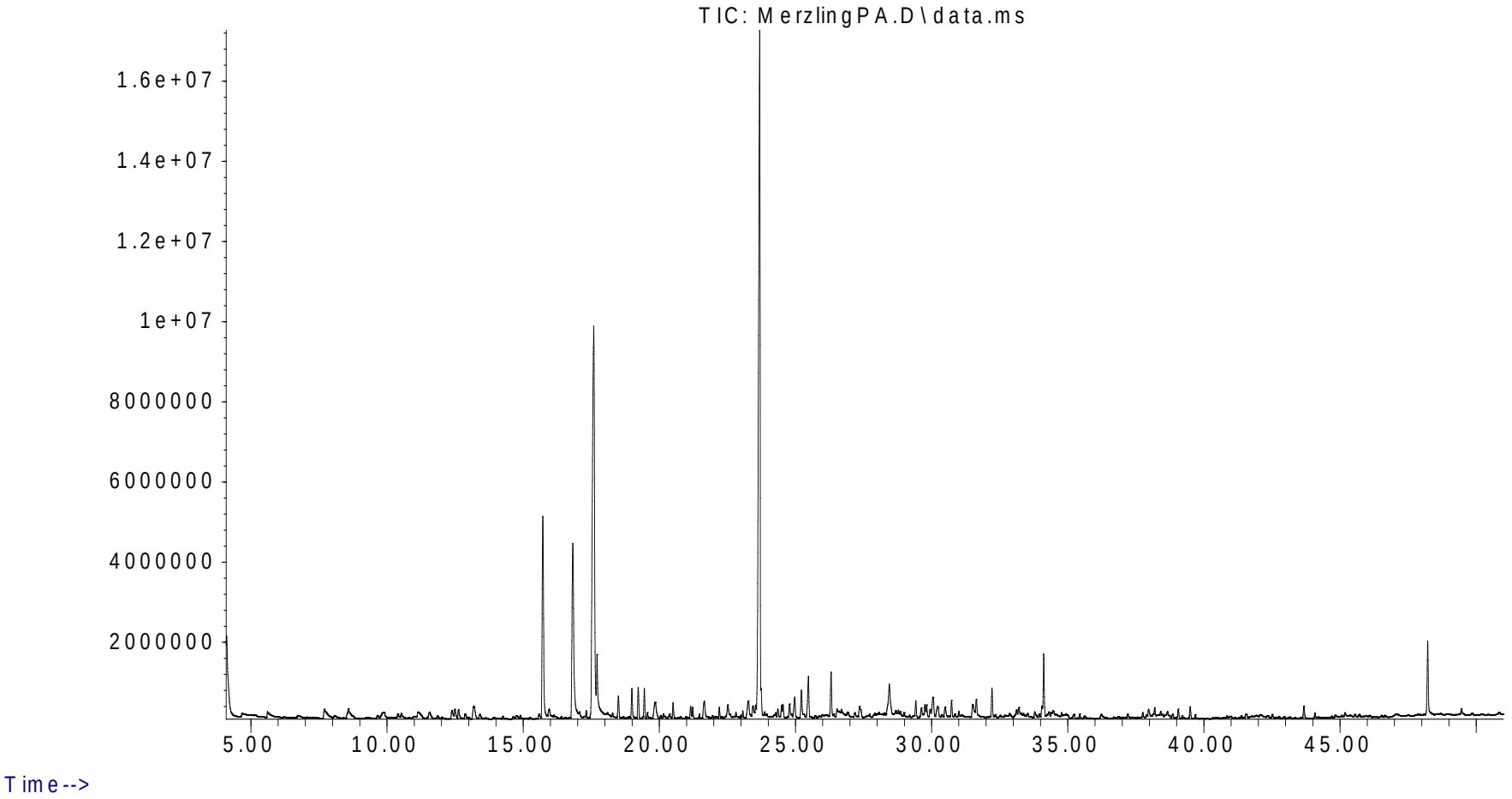

Figure 21 – GC-MS chromatogram of Merzling grapes extract after acid hydrolysis.

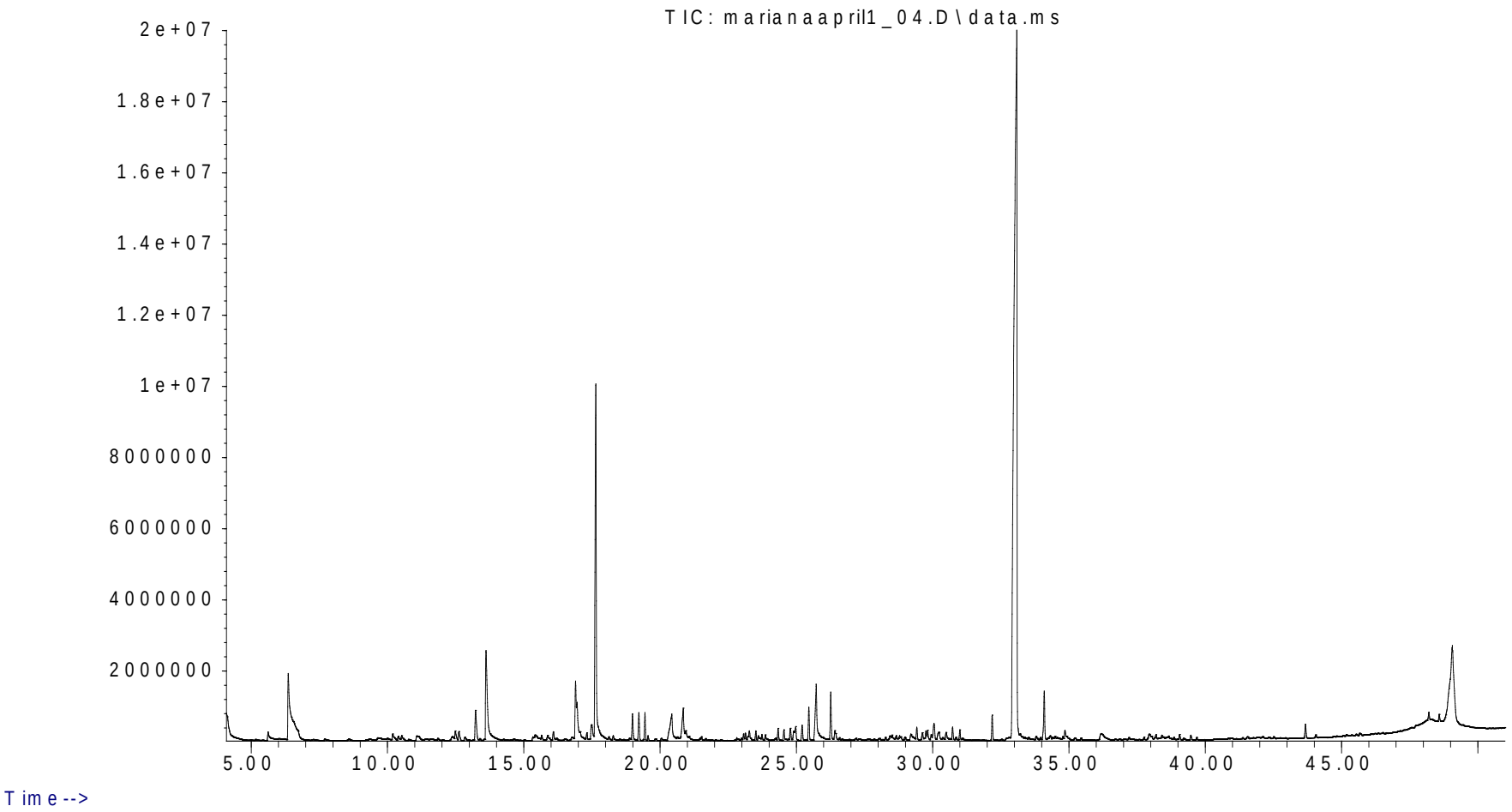

Figure 22 – GC-MS chromatogram of Freiminer grapes non–polar extract.

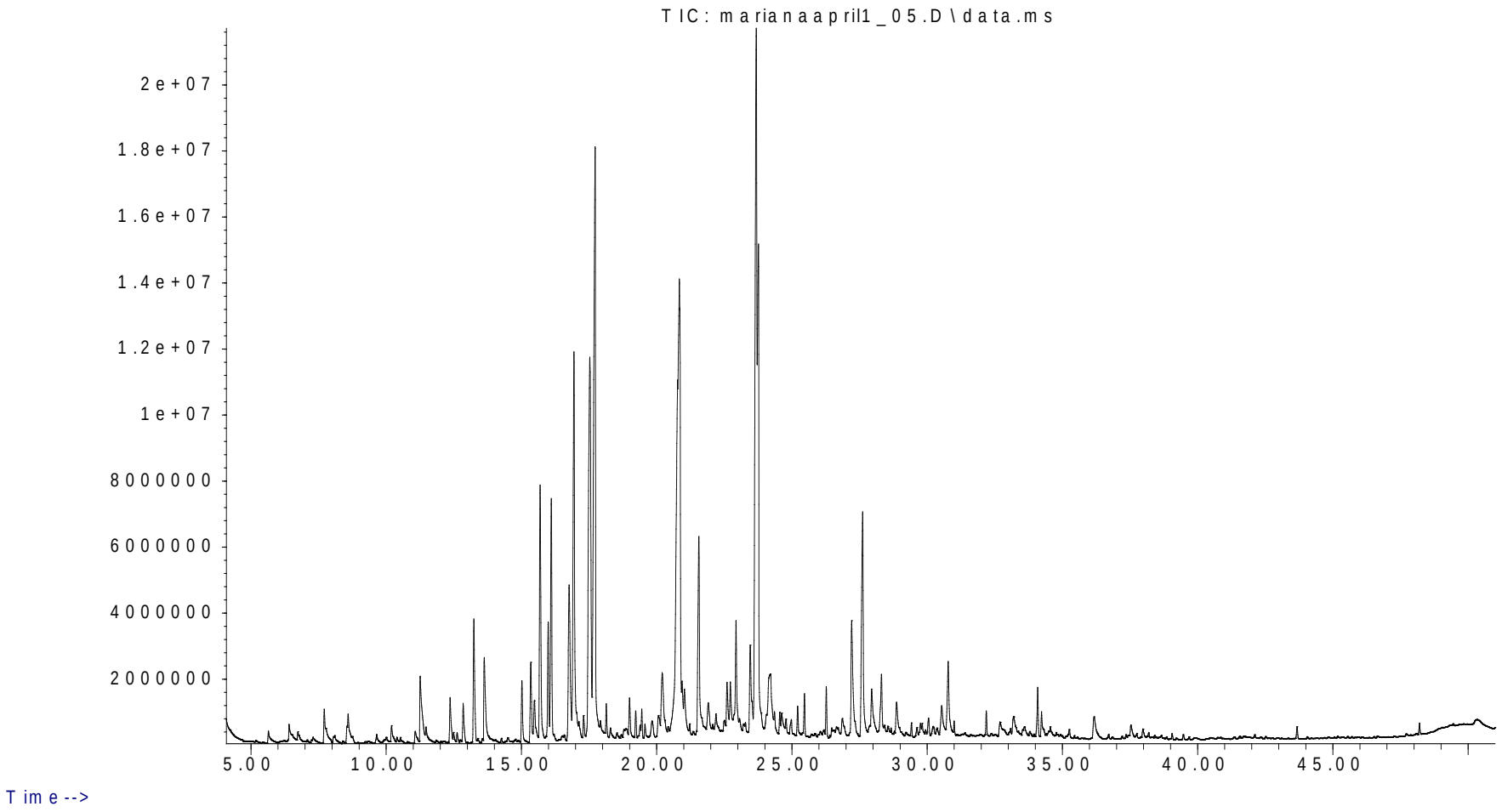

Figure 23 – GC-MS chromatogram of Freiminer grapes extract after enzymatic hydrolysis.

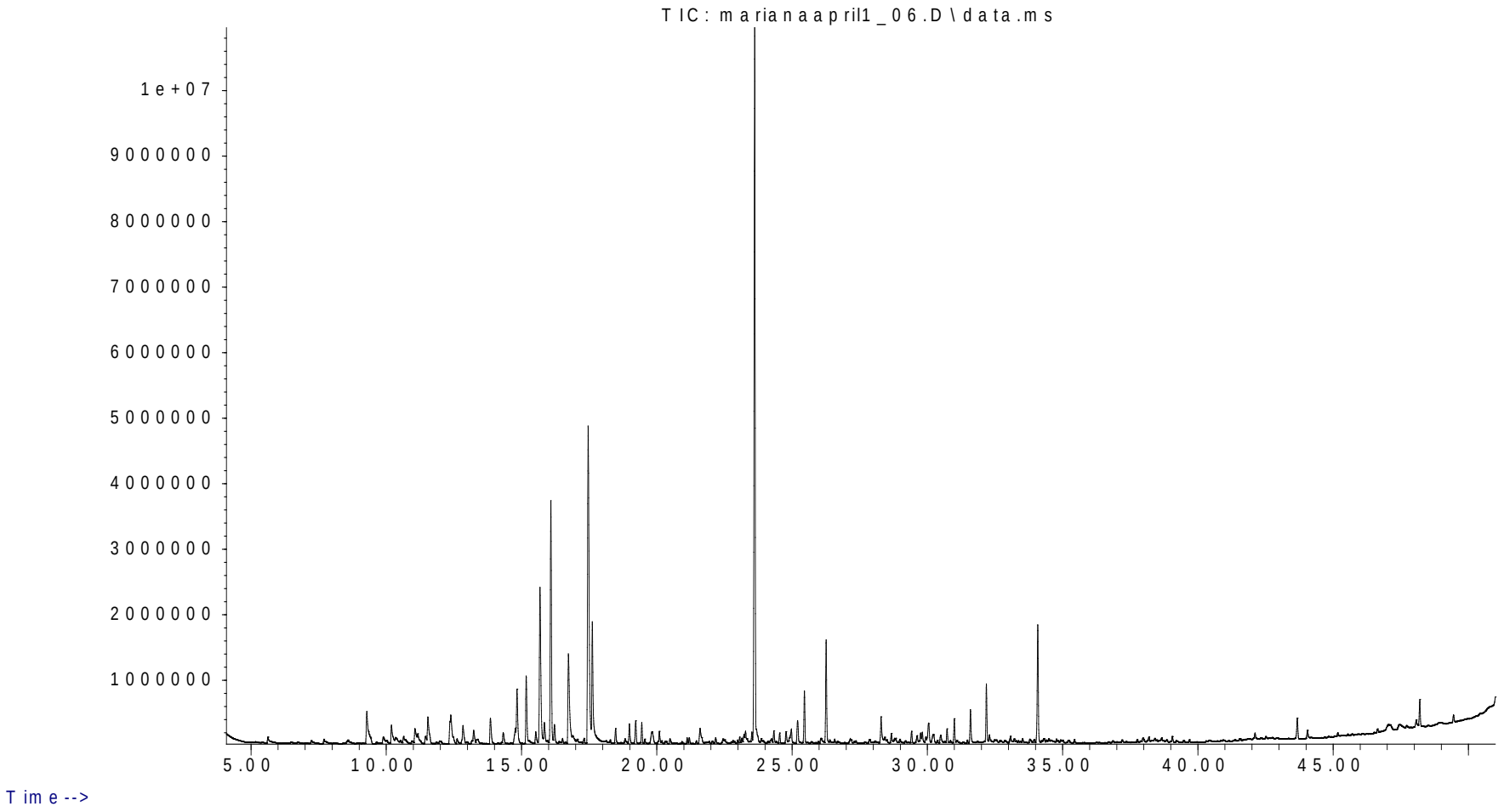

Figure 24 – GC-MS chromatogram of Freiminer grapes extract after acid hydrolysis.

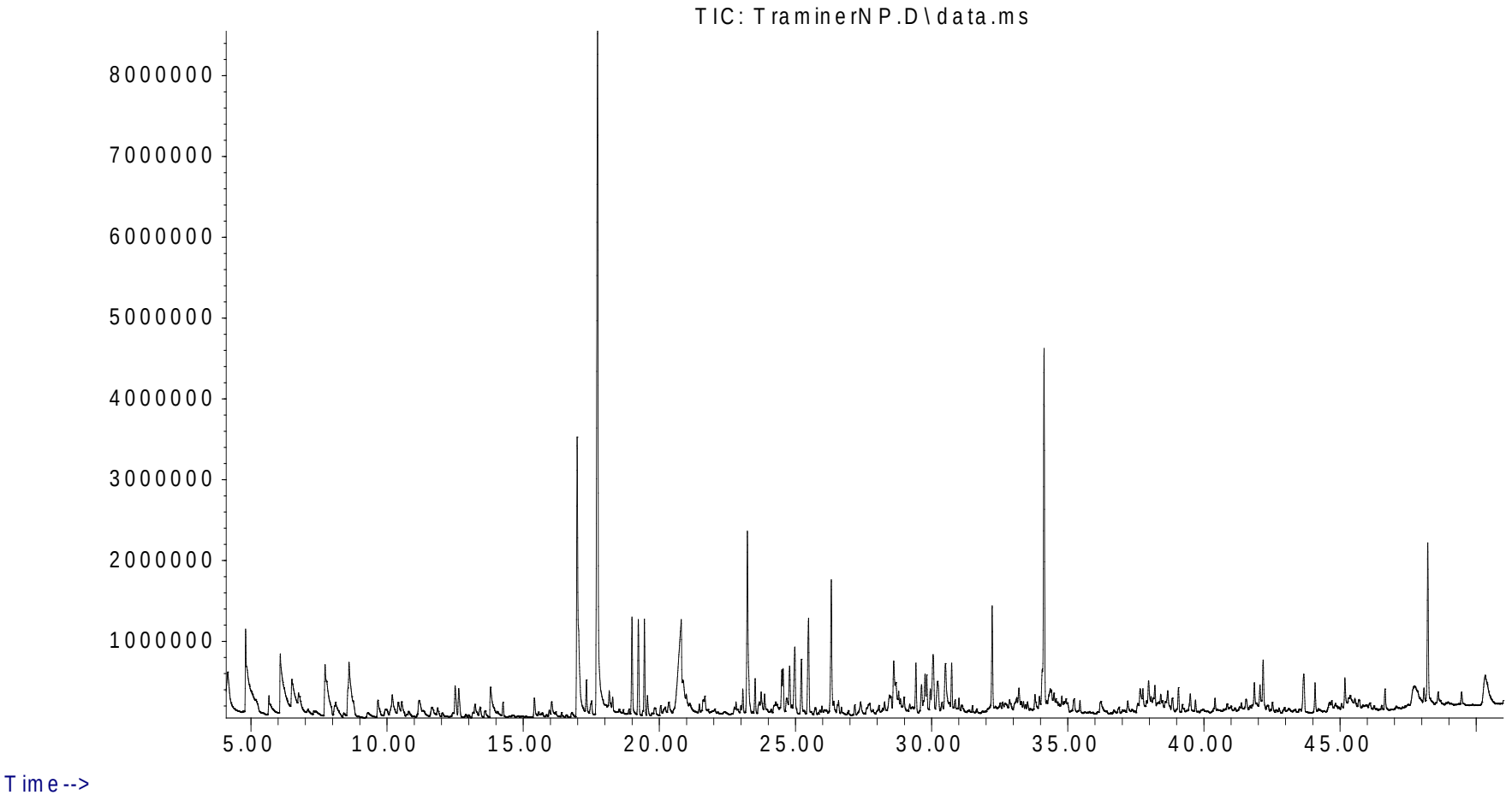

Figure 25 – GC-MS chromatogram of Traminer grapes non–polar extract.

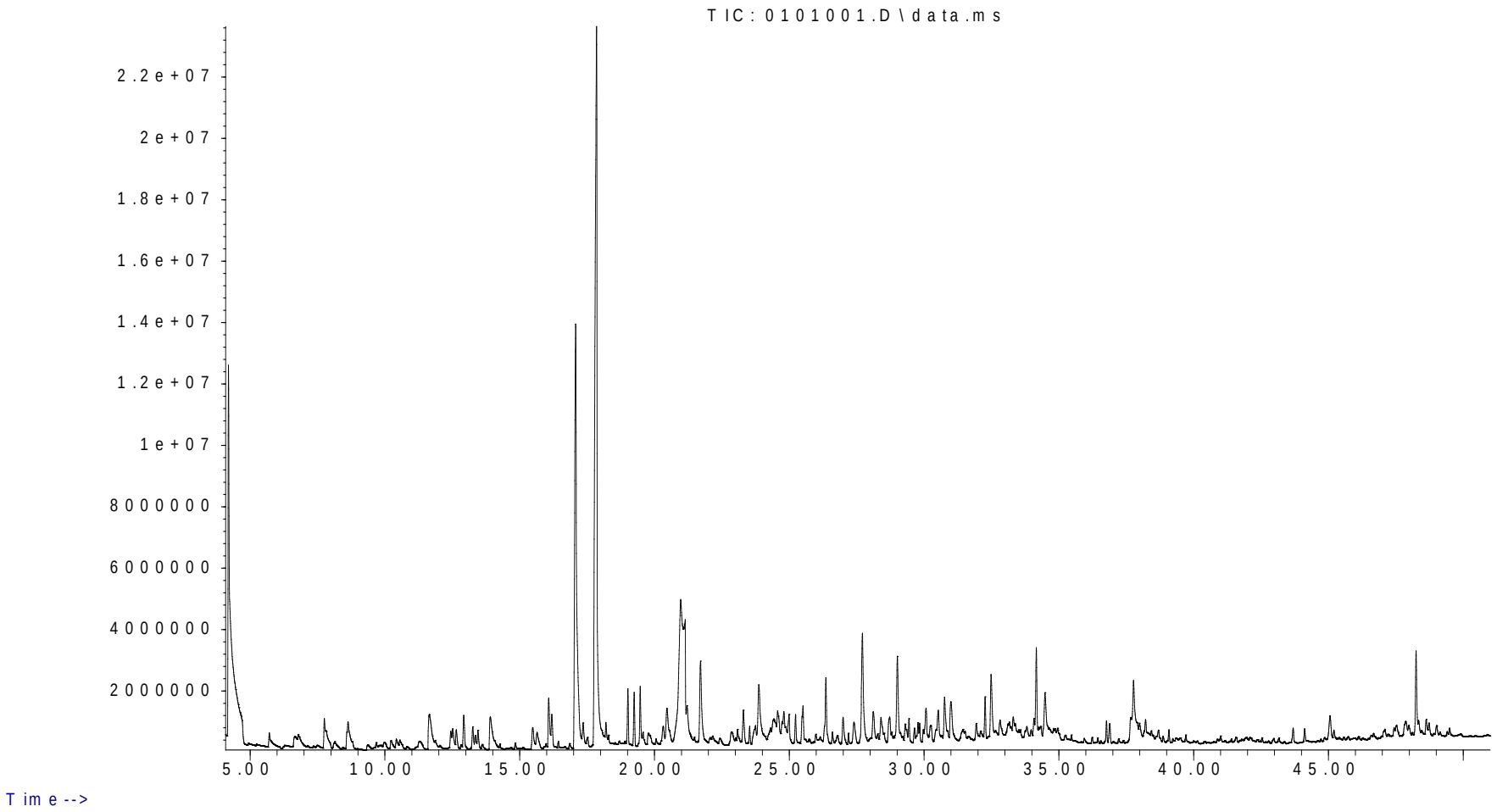

Figure 26 – GC-MS chromatogram of Traminer grapes extract after enzymatic hydrolysis.

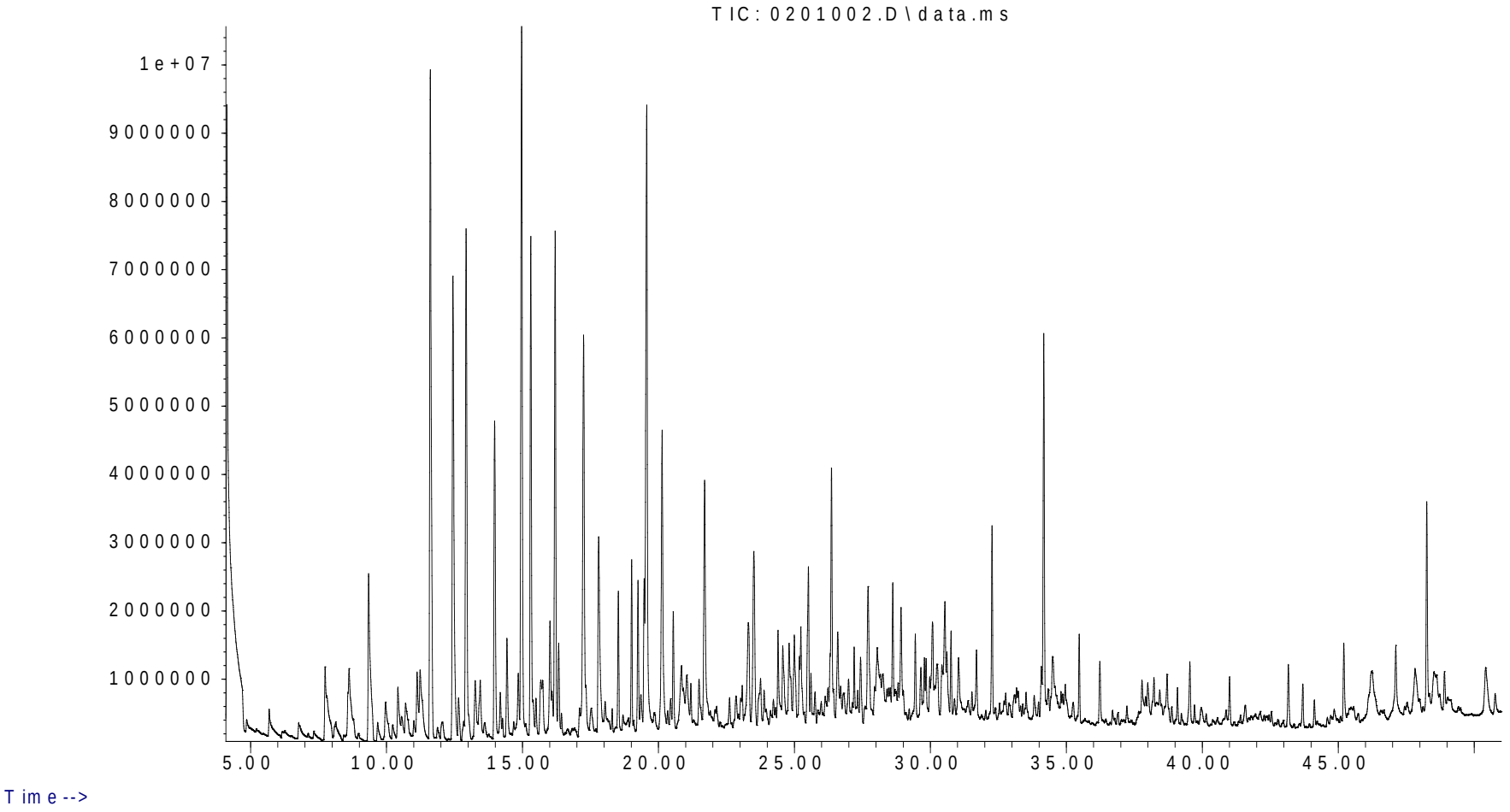

Figure 27 – GC-MS chromatogram of Traminer grapes extract after acid hydrolysis.

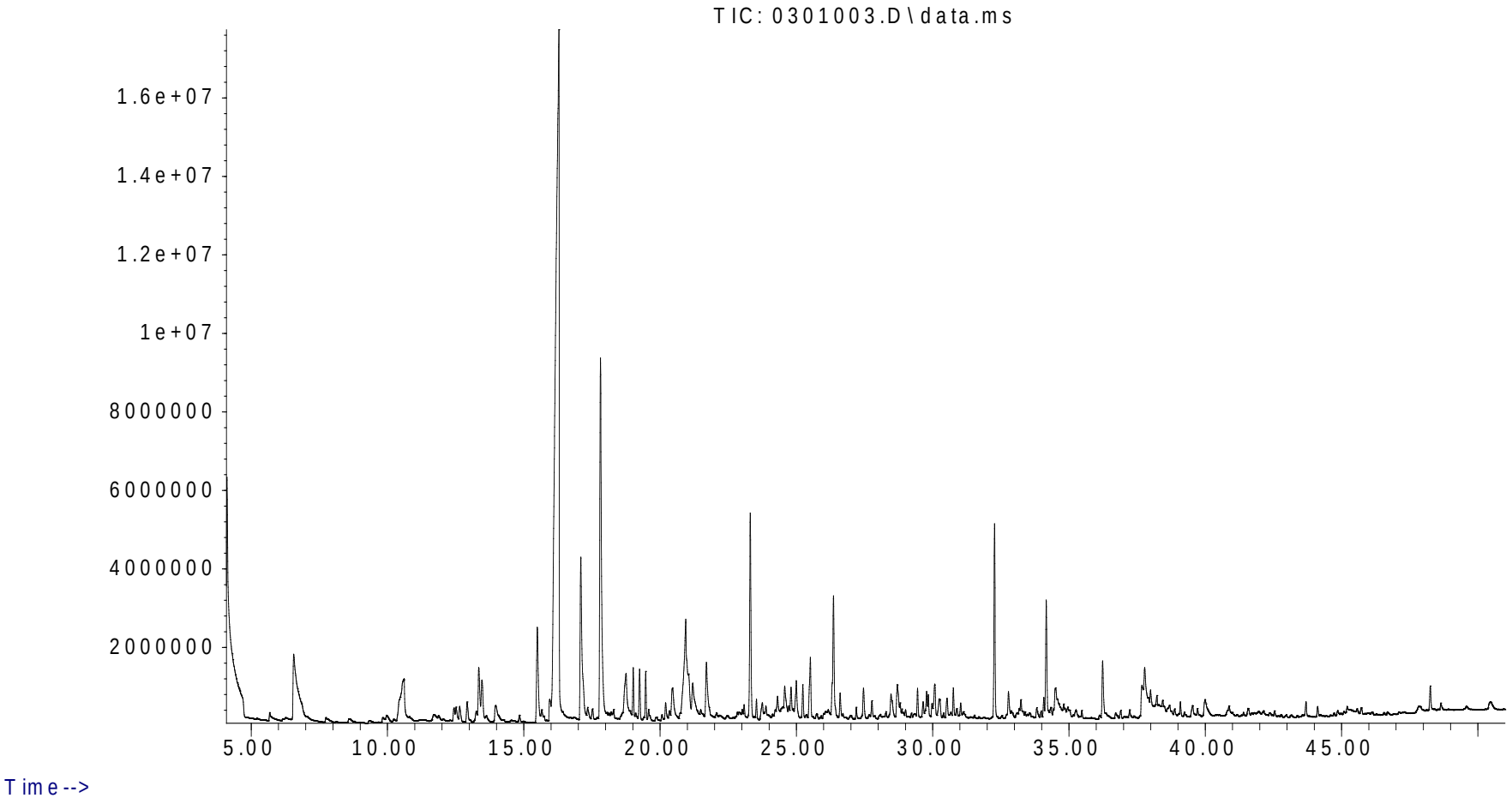

Figure 28 – GC-MS chromatogram of Jutrzenka grapes non–polar extract.

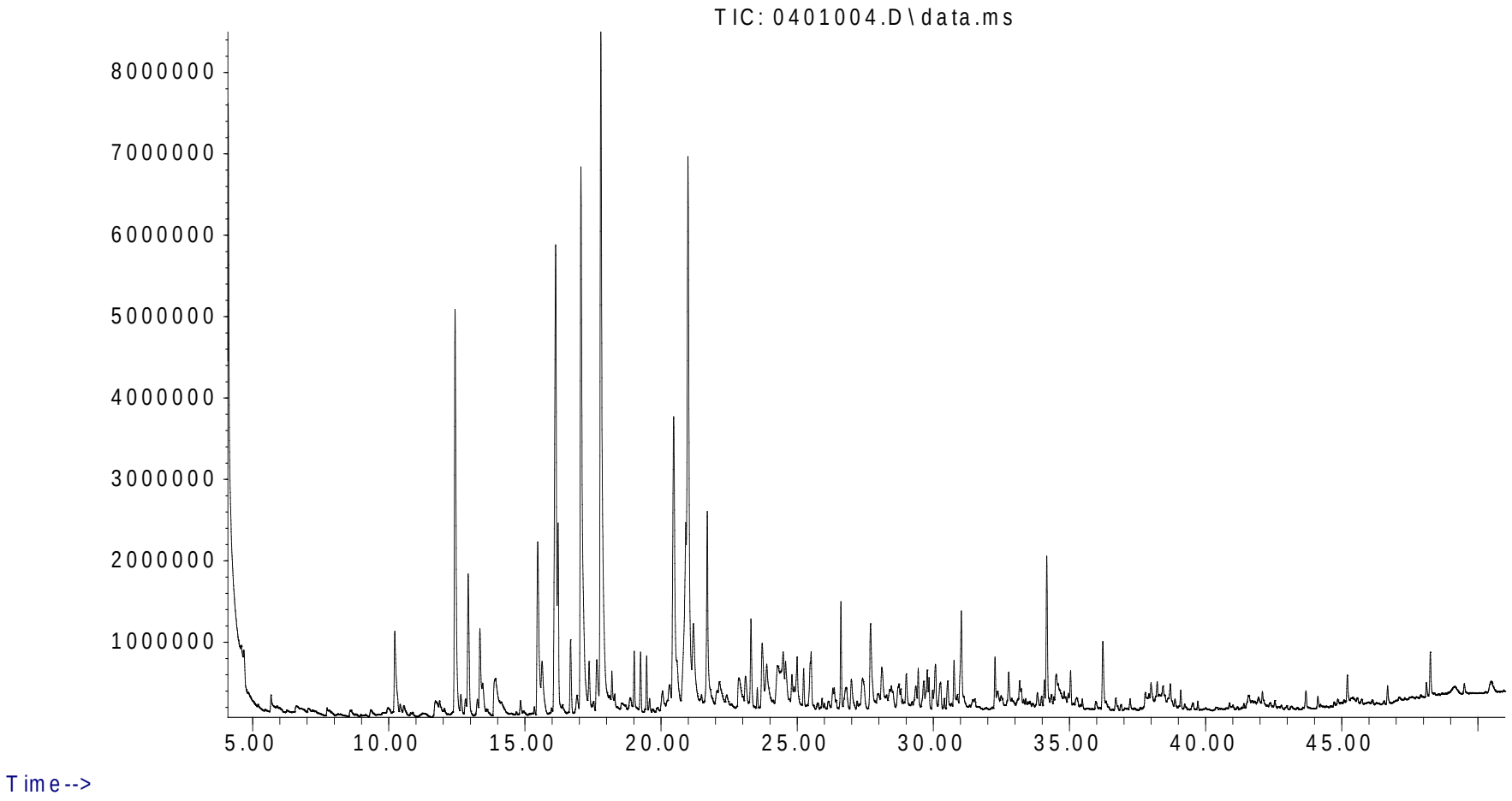

Figure 29 – GC-MS chromatogram of Jutrzenka grapes extract after enzymatic hydrolysis.

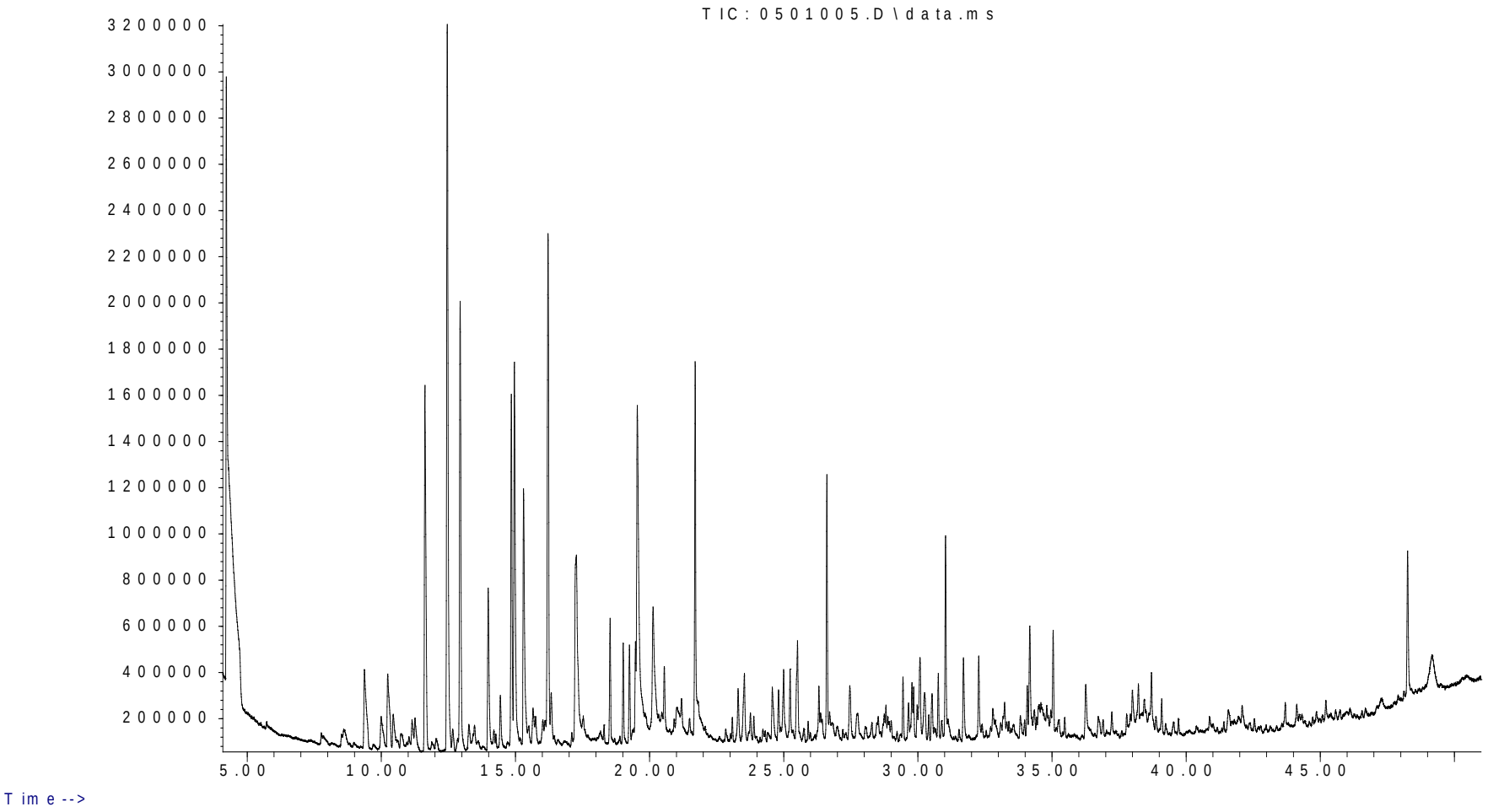

Figure 30 – GC-MS chromatogram of Jutrzenka grapes extract after acid hydrolysis.

– o –

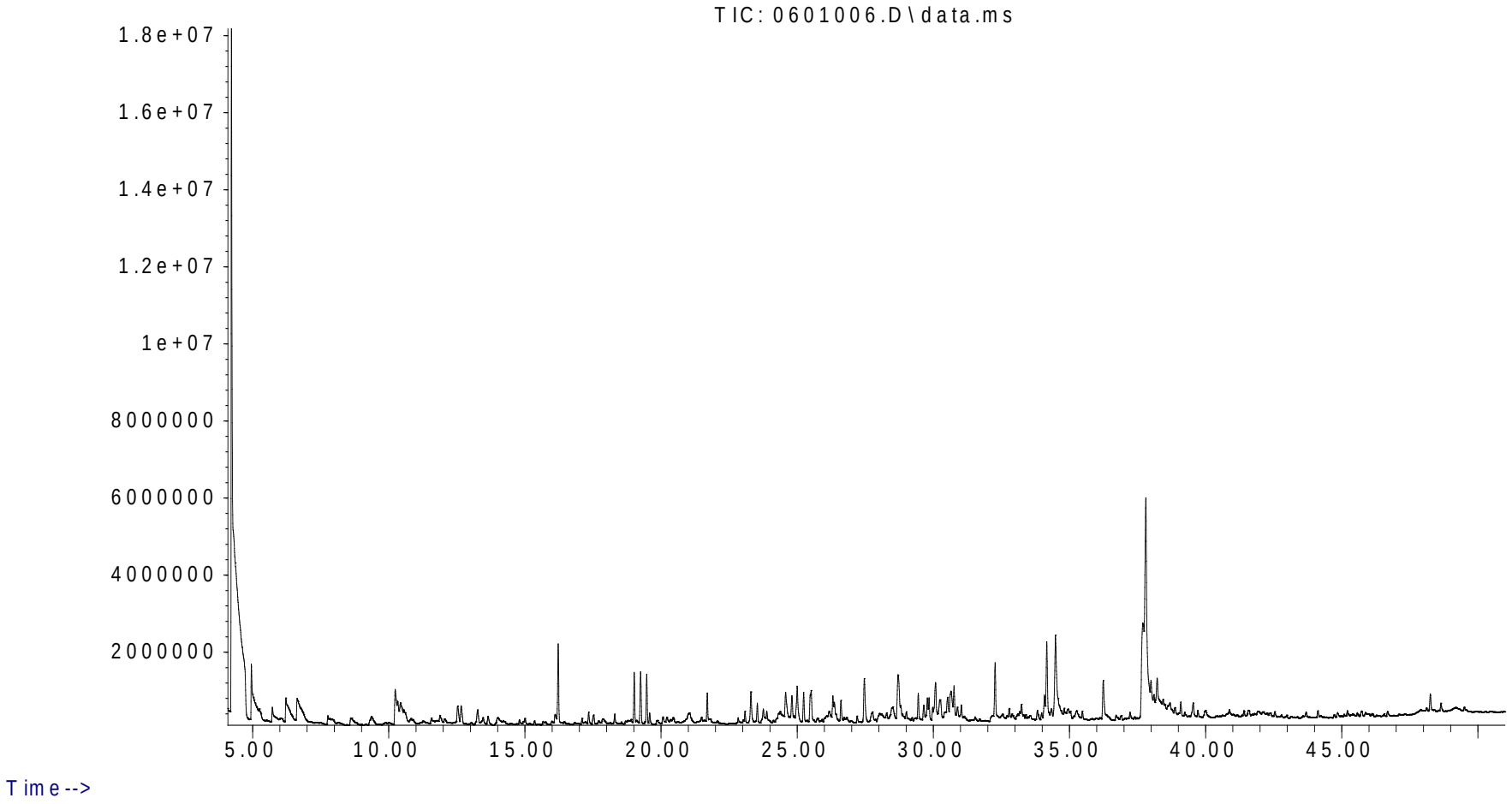

Figure 31 – GC-MS chromatogram of Adalmiina grapes non–polar extract.

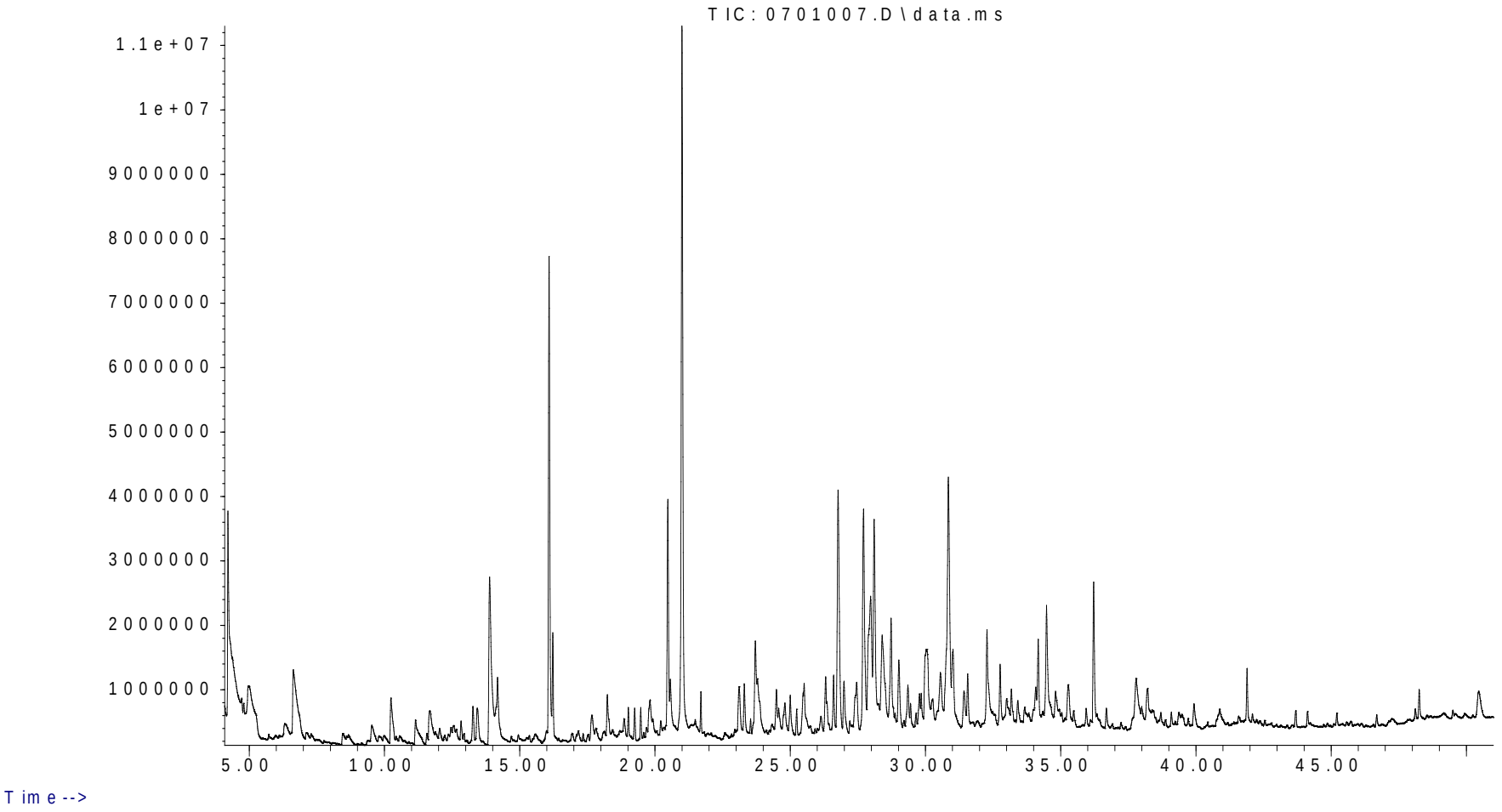

Figure 32 – GC-MS chromatogram of Adalmiina grapes extract after enzymatic hydrolysis.

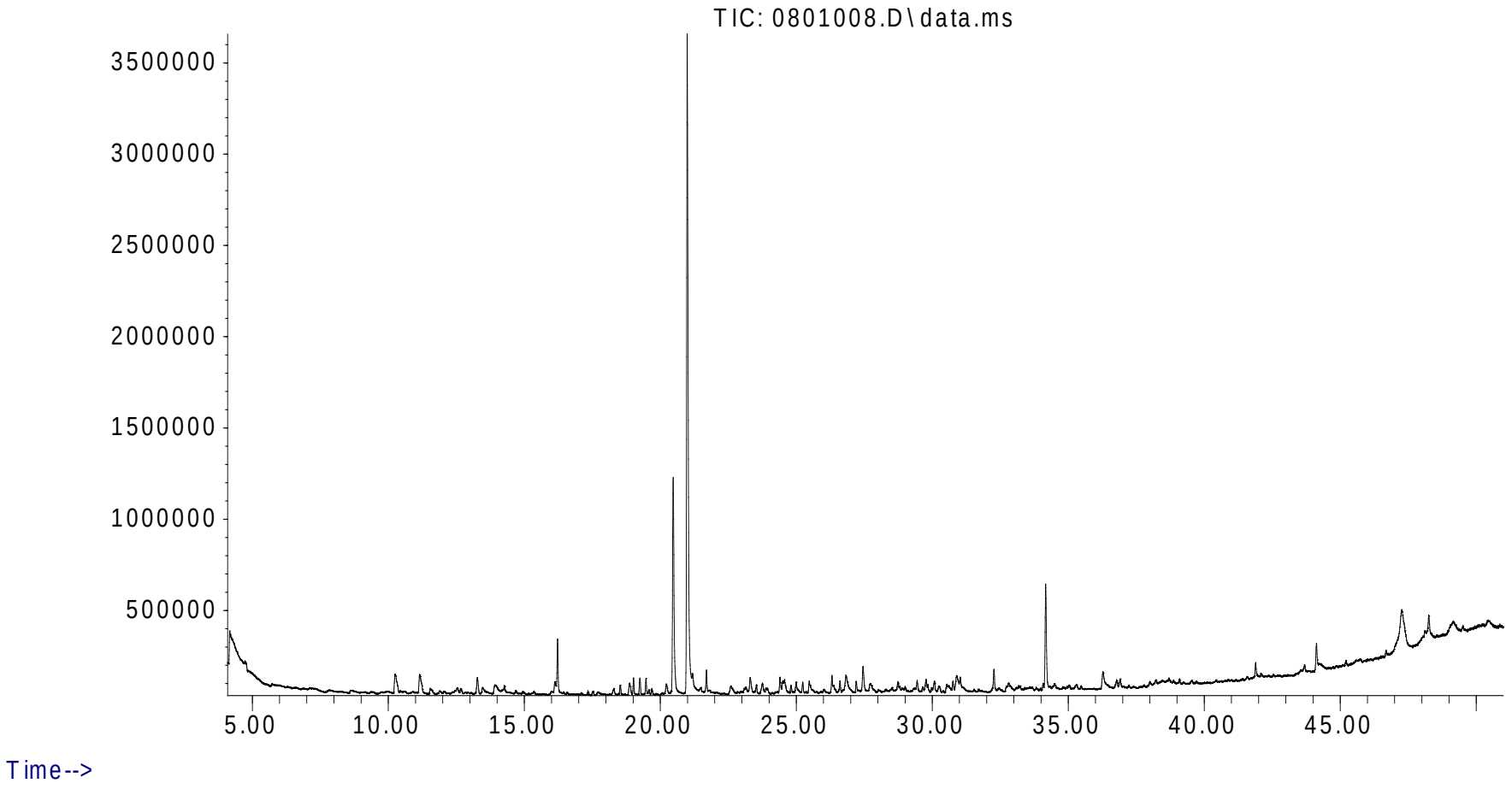

Figure 33 – GC-MS chromatogram of Adalmiina grapes extract after acid hydrolysis.